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(54) Title: 57250, A NOVEL HUMAN SUGAR TRANSPORTER FAMILY MEMBER AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acid molecules, designated HST-1 nucleic acid molecules, which encode novel sugar transporter family molecules. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing HST-1 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which an HST-1 gene has been introduced or disrupted. The invention still further provides isolated HST-1 polypeptides, fusion polypeptides, antigenic peptides and anti-HST-1 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

57250, A NOVEL HUMAN SUGAR TRANSPORTER FAMILY MEMBER AND USES THEREOF

Related Applications

This application claims priority to U.S. Provisional Patent Application No. 60/221,769 filed on July 31, 2000, incorporated herein in its entirety by reference.

Background of the Invention

Cellular membranes serve to differentiate the contents of a cell from the surrounding environment, and may also serve as effective barriers against the unregulated influx of hazardous or unwanted compounds, and the unregulated efflux of desirable compounds. Membranes are by nature impervious to the unfacilitated diffusion of hydrophilic compounds such as proteins, water molecules, and ions due to their structure: a bilayer of lipid molecules in which the polar head groups face outward (towards the exterior and interior of the cell) and the nonpolar tails face inward (at the center of bilayer, forming a hydrophobic core). Membranes enable a cell to maintain a relatively higher intracellular concentration of desired compounds and a relatively lower intracellular concentration of undesired compounds than are contained within the surrounding environment.

Membranes also present a structural difficulty for cells, in that most desired compounds cannot readily enter the cell, nor can most waste products readily exit the cell through this lipid bilayer. The import and export of such compounds is regulated by proteins which are embedded (singly or in complexes) in the cellular membrane. Two mechanisms exist whereby membrane proteins allow the passage of compounds: non-mediated and mediated transport. Simple diffusion is an example of non-mediated transport, while facilitated diffusion and active transport are examples of mediated transport. Permeases, porters, translocases, translocators, and transporters are proteins that engage in mediated transport (Voet and Voet (1990) Biochemistry, John Wiley and Sons, Inc., New York, N.Y. pp. 484-505).

Sugar transporters are members of the major facilitator superfamily of transporters. These transporters are passive in the sense that they are driven by the substrate concentration gradient and they exhibit distinct kinetics as well as sugar substrate specificity. Members of this family share several characteristics: (1) they contain twelve transmembrane domains separated by hydrophilic loops; (2) they have intracellular N- and C-termini; and (3) they are thought to function as oscillating pores. The transport mechanism occurs via sugar binding to the exofacial binding site of the transporter, which is thought to trigger a conformational change causing the sugar binding site to re-orient to the endofacial conformation, allowing the release of substrate. These transporters are specific for various sugars and are found in both prokaryotes and eukaryotes. In mammals, sugar

transporters transport various monosaccharides across the cell membrane (Walmsley *et al.* (1998) *Trends in Biochem. Sci.* 23:476-481; Barrett *et al.* (1999) *Curr. Op. Cell Biol.* 11:496-502).

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel human sugar transporter family members, referred to herein as "human sugar transporter-1" or "HST-1" nucleic acid and polypeptide molecules. The HST-1 nucleic acid and polypeptide molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, *e.g.*, sugar homeostasis. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding HST-1 polypeptides or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of HST-1-encoding nucleic acids.

In one embodiment, the invention features an isolated nucleic acid molecule that includes the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3. In another embodiment, the invention features an isolated nucleic acid molecule that encodes a polypeptide including the amino acid sequence set forth in SEQ ID NO:2. In another embodiment, the invention features an isolated nucleic acid molecule that includes the nucleotide sequence contained in the plasmid deposited with ATCC® as Accession Number _____.

In still other embodiments, the invention features isolated nucleic acid molecules including nucleotide sequences that are substantially identical (*e.g.*, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical) to the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. The invention further features isolated nucleic acid molecules including at least 50, 57, 63, 72, 100, 124, 150, 172, 175, 200, 250, 268, 300, 305, 328, 350, 400, 431, 450, 495, 500, 550, 600, 650, 700, 750, 800, 804, 850, 900, 950, 1000, 1050, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900 or more contiguous nucleotides of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. In another embodiment, the invention features isolated nucleic acid molecules which encode a polypeptide including an amino acid sequence that is substantially identical (*e.g.*, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical) to the amino acid sequence set forth as SEQ ID NO:2. The present invention also features nucleic acid molecules which encode allelic variants of the polypeptide having the amino acid sequence set forth as SEQ ID NO:2. In addition to isolated nucleic acid molecules encoding full-length polypeptides, the present invention also features nucleic acid molecules which encode fragments, for example, biologically active or antigenic fragments, of the full-length polypeptides of the present invention (*e.g.*, fragments including at least 10, 20, 50, 100, 150, 155, 200, 250, 300,

350, 350, 400, 450, 500 or more contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2). In still other embodiments, the invention features nucleic acid molecules that are complementary to, antisense to, or hybridize under stringent conditions to the isolated nucleic acid molecules described herein.

In another aspect, the invention provides vectors including the isolated nucleic acid molecules described herein (*e.g.*, HST-1-encoding nucleic acid molecules). Such vectors can optionally include nucleotide sequences encoding heterologous polypeptides. Also featured are host cells including such vectors (*e.g.*, host cells including vectors suitable for producing HST-1 nucleic acid molecules and polypeptides).

In another aspect, the invention features isolated HST-1 polypeptides and/or biologically active or antigenic fragments thereof. Exemplary embodiments feature a polypeptide including the amino acid sequence set forth as SEQ ID NO:2, a polypeptide including an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence set forth as SEQ ID NO:2, a polypeptide encoded by a nucleic acid molecule including a nucleotide sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3. Also featured are fragments of the full-length polypeptides described herein (*e.g.*, fragments including at least 10, 20, 50, 100, 150, 155, 200, 250, 300, 350, 350, 400, 450, 500 or more contiguous amino acid residues of the sequence set forth as SEQ ID NO:2) as well as allelic variants of the polypeptide having the amino acid sequence set forth as SEQ ID NO:2.

The HST-1 polypeptides and/or biologically active or antigenic fragments thereof, are useful, for example, as reagents or targets in assays applicable to treatment and/or diagnosis of HST-1 mediated or related disorders. In one embodiment, an HST-1 polypeptide or fragment thereof, has an HST-1 activity. In another embodiment, an HST-1 polypeptide or fragment thereof, has a transmembrane domain and/or a sugar transporter family domain, and optionally, has an HST-1 activity. In a related aspect, the invention features antibodies (*e.g.*, antibodies which specifically bind to any one of the polypeptides described herein) as well as fusion polypeptides including all or a fragment of a polypeptide described herein.

The present invention further features methods for detecting HST-1 polypeptides and/or HST-1 nucleic acid molecules, such methods featuring, for example, a probe, primer or antibody described herein. Also featured are kits *e.g.*, kits for the detection of HST-1 polypeptides and/or HST-1 nucleic acid molecules. In a related aspect, the invention features methods for identifying compounds which bind to and/or modulate the activity of an HST-1 polypeptide or HST-1 nucleic acid molecule described herein. Further featured are methods for modulating an HST-1 activity.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figures 1A-1B depict the cDNA sequence and predicted amino acid sequence of human HST-1. The nucleotide sequence corresponds to nucleic acids 1 to 1917 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 572 of SEQ ID NO: 2. The coding region without the 5' and 3' untranslated regions of the human HST-1 gene is shown in SEQ ID NO:3.

Figure 2 depicts a structural, hydrophobicity, and antigenicity analysis of the human HST-1 polypeptide.

Figure 3 depicts the results of a search which was performed against the HMM database in PFAM and which resulted in the identification of one "sugar transporter family domain" in the human HST-1 polypeptide (SEQ ID NO:2).

Figure 4 depicts the results of a search which was performed against the MEMSAT database and which resulted in the identification of twelve "transmembrane domains" in the human HST-1 polypeptide (SEQ ID NO:2).

Figure 5 depicts an alignment of the human HST-1 amino acid sequence (SEQ ID NO:2) with the amino acid sequence of a human potent brain type organic ion transporter (Accession No. AB040056) using the CLUSTAL W (1.74) alignment program.

Figure 6 is a graph depicting the expression of human HST-1 cDNA (SEQ ID NO:2) in various human tissues as determined by Taqman analysis.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as "human sugar transporter-1" or "HST-1" nucleic acid and polypeptide molecules, which are novel members of the sugar transporter family. These novel molecules are capable of, for example, modulating a transporter mediated activity (*e.g.*, a sugar transporter mediated activity) in a cell, *e.g.*, a liver cell, fat cell, muscle cell, or blood cell, such as an erythrocyte. These novel molecules are capable of transporting molecules, *e.g.*, monosaccharides such as D-glucose, D-fructose or D-galactose, across biological membranes and, thus, play a role in or function in a variety of cellular processes, *e.g.*, maintenance of sugar homeostasis.

As used herein, a "sugar transporter" includes a protein or polypeptide which is involved in transporting a molecule, *e.g.*, a monosaccharide such as D-glucose, D-fructose or D-galactose, across the plasma membrane of a cell, *e.g.*, a liver cell, fat cell, muscle cell, or blood cell, such as an erythrocyte. Sugar transporters regulate sugar homeostasis in a cell

and, typically, have sugar substrate specificity. Examples of sugar transporters include glucose transporters, fructose transporters, and galactose transporters.

As used herein, a "sugar transporter mediated activity" includes an activity which involves a sugar transporter, *e.g.*, a sugar transporter in a liver cell, fat cell, muscle cell, or blood cell, such as an erythrocyte. Sugar transporter mediated activities include the transport of sugars, *e.g.*, D-glucose, D-fructose or D-galactose, into and out of cells; the stimulation of molecules that regulate glucose homeostasis (*e.g.*, insulin and glucagon), in cells, *e.g.*, pancreatic cells; and the participation in signal transduction pathways associated with sugar metabolism.

As the HST-1 molecules of the present invention are sugar transporters, they may be useful for developing novel diagnostic and therapeutic agents for sugar transporter associated disorders. As used herein, the term "sugar transporter associated disorder" includes a disorder, disease, or condition which is characterized by an aberrant, *e.g.*, upregulated or downregulated, sugar transporter mediated activity. Sugar transporter associated disorders typically result in, for example, upregulated or downregulated, sugar levels in a cell. Examples of sugar transporter associated disorders include disorders associated with sugar homeostasis, such as obesity, anorexia, type-1 diabetes, type-2 diabetes, hypoglycemia, glycogen storage disease (Von Gierke disease), type I glycogenosis, bipolar disorder, seasonal affective disorder, and cluster B personality disorders. HST-1-associated disorders may also include cellular growth or proliferation disorders. Further examples of sugar transporter associated disorders include cellular growth or proliferation disorders, such as cancer, *e.g.*, carcinoma, sarcoma, or leukemia, examples of which include, but are not limited to, colon, ovarian, lung, breast, endometrial, uterine, hepatic, gastrointestinal, prostate, and brain cancer; tumorigenesis and metastasis; skeletal dysplasia; and hematopoietic and/or myeloproliferative disorders.

The term "family" when referring to the polypeptide and nucleic acid molecules of the invention is intended to mean two or more polypeptides or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first polypeptide of human origin, as well as other, distinct polypeptides of human origin or alternatively, can contain homologues of non-human origin, *e.g.*, mouse or monkey polypeptides. Members of a family may also have common functional characteristics.

For example, the family of HST-1 polypeptides comprise at least one "transmembrane domain" and preferably twelve transmembrane domains. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 20-45 amino acid residues in length which spans the plasma membrane. More preferably, a

transmembrane domain includes about at least 20, 25, 30, or 35 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an alpha-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, alanines, valines, phenylalanines, prolines or methionines. Transmembrane domains are described in, for example, Zagotta W.N. *et al,* (1996) *Annual Rev. Neurosci.* 19: 235-263, the contents of which are incorporated herein by reference. A MEMSAT analysis resulted in the identification of twelve transmembrane domains in the amino acid sequence of human HST-1 (SEQ ID NO:2) at about residues 20-36, 150-167, 174-196, 204-220, 231-255, 263-282, 355-372, 387-405, 413-431, 438-462, 469-485, and 505-521 as set forth in Figure 4.

Accordingly, HST -1 polypeptides having at least 50-60% homology, preferably about 60-70%, more preferably about 70-80%, or about 80-90% homology with a transmembrane domain of human HST -1 are within the scope of the invention.

In another embodiment, an HST-1 molecule of the present invention is identified based on the presence of at least one "sugar transporter family domain." As used herein, the term "sugar transporter family domain" includes a protein domain having at least about 350-500 amino acid residues and a sugar transporter mediated activity. Preferably, a sugar transporter family domain includes a polypeptide having an amino acid sequence of about 350-450, 400-450, or more preferably, about 419 amino acid residues and a sugar transporter mediated activity. To identify the presence of a sugar transporter family domain in an HST-1 protein, and make the determination that a protein of interest has a particular profile, the amino acid sequence of the protein may be searched against a database of known protein domains (*e.g.*, the PFAM HMM database). A PFAM sugar transporter family domain has been assigned the PFAM Accession PF00083. A search was performed against the PFAM HMM database resulting in the identification of a sugar transporter family domain in the amino acid sequence of human HST-1 (SEQ ID NO:2) at about residues 117-536 of SEQ ID NO:2. The results of the search are set forth in Figure 3.

Preferably a "sugar transporter family domain" has a "sugar transporter mediated activity" as described herein. For example, a sugar transporter family domain may have the ability to bind a monosaccharide, such as D-glucose, D-fructose, and/or D-galactose; the ability to transport a monosaccharide such as D-glucose, D-fructose, and/or D-galactose, across a cell membrane (*e.g.*, a liver cell membrane, fat cell membrane, muscle cell membrane, and/or blood cell membrane, such as an erythrocyte membrane); and the ability to modulate sugar homeostasis in a cell. Accordingly, identifying the presence of a "sugar transporter family domain" can include isolating a fragment of an HST-1 molecule (*e.g.*, an HST-1 polypeptide) and assaying for the ability of the fragment to exhibit one of the aforementioned sugar transporter mediated activities.

A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28:405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.*(1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.*(1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.*(1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.*(1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference.

In a preferred embodiment, the NPM-1 molecules of the invention include at least one, preferably two, even more preferably twelve transmembrane domain(s) and/or at least one sugar transporter family domain.

Isolated polypeptides of the present invention, preferably HST-1 polypeptides, have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or are encoded by a nucleotide sequence sufficiently identical to SEQ ID NO:1 or 3. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (*e.g.*, an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences share common structural domains or motifs and/or a common functional activity. For example, amino acid or nucleotide sequences which share common structural domains having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homology or identity across the amino acid sequences of the domains and contain at least one and preferably two structural domains or motifs, are defined herein as sufficiently identical. Furthermore, amino acid or nucleotide sequences which share at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homology or identity and share a common functional activity are defined herein as sufficiently identical.

In a preferred embodiment, an HST-1 polypeptide includes at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain, and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous or identical to the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In yet another preferred embodiment, an HST-1 polypeptide includes at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain, and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3. In another preferred embodiment, an HST-1 polypeptide includes at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain, and has an HST-1 activity.

As used interchangeably herein, an "HST-1 activity", "biological activity of HST-1" or "functional activity of HST-1," refers to an activity exerted by an HST-1 polypeptide or nucleic acid molecule on an HST-1 responsive cell or tissue, or on an HST-1 polypeptide substrate, as determined *in vivo*, or *in vitro*, according to standard techniques. In one embodiment, an HST-1 activity is a direct activity, such as an association with an HST-1-target molecule. As used herein, a "substrate," "target molecule," or "binding partner" is a molecule with which an HST-1 polypeptide binds or interacts in nature, such that HST-1-mediated function is achieved. An HST-1 target molecule can be a non-HST-1 molecule or an HST-1 polypeptide or polypeptide of the present invention. In an exemplary embodiment, an HST-1 target molecule is an HST-1 ligand, *e.g.*, a sugar transporter ligand such as D-glucose, D-fructose, and/or D-galactose. Alternatively, an HST-1 activity is an indirect activity, such as a cellular signaling activity mediated by interaction of the HST-1 polypeptide with an HST-1 ligand. The biological activities of HST-1 are described herein. For example, the HST-1 polypeptides of the present invention can have one or more of the following activities: (1) maintain sugar homeostasis in a cell, (2) influence insulin and/or glucagon secretion, (3) bind a monosaccharide, *e.g.*, D-glucose, D-fructose, and/or D-galactose, and/or (4) transport monosaccharides across a cell membrane.

The nucleotide sequence of the isolated human HST-1 cDNA and the predicted amino acid sequence of the human HST-1 polypeptide are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively. A plasmid containing the nucleotide sequence encoding human HST-1 was deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The human HST-1 gene, which is approximately 1917 nucleotides in length, encodes a polypeptide which is approximately 572 amino acid residues in length.

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode HST-1 polypeptides or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify HST-1-encoding nucleic acid molecules (*e.g.*, HST-1 mRNA) and fragments for use as PCR primers for the amplification or mutation of HST-1 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA

molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated HST-1 nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, as a hybridization probe, HST-1 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis.

Furthermore, oligonucleotides corresponding to HST-1 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

In one embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1. The sequence of SEQ ID NO:1 corresponds to the human HST-1 cDNA. This cDNA comprises sequences encoding the human HST-1 polypeptide (*i.e.*, "the coding region", from nucleotides 13-1732) as well as 5' untranslated sequences (nucleotides 1-12) and 3' untranslated sequences (nucleotides 1733-1917). Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 13-1732, corresponding to SEQ ID NO:3). Accordingly, in another embodiment, the isolated nucleic acid molecule comprises SEQ ID NO:3 and nucleotides 1-12 and 1733-1917 of SEQ ID NO:1. In yet another embodiment, the nucleic acid molecule consists of the nucleotide sequence set forth as SEQ ID NO:1 or SEQ ID NO:3.

In still another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ , or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence shown in SEQ ID NO:1 or 3 (*e.g.*, to the entire length of the nucleotide sequence), or to the nucleotide sequence (*e.g.*, the entire length of the nucleotide sequence) of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion of any of these nucleotide sequences. In one embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least (or no greater than) 50, 57, 63, 72, 100, 124, 150, 172, 175, 200, 250, 268, 300, 305, 328, 350, 400, 431, 450, 495, 500, 550, 600, 650, 700, 750, 800, 804, 850, 900, 950, 1000, 1050, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900 or more nucleotides in length and hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule of SEQ ID NO:1 or 3,

or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of an HST-1 polypeptide, *e.g.*, a biologically active portion of an HST-1 polypeptide. The nucleotide sequence determined from the cloning of the HST-1 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other HST-1 family members, as well as HST-1 homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide. The probe/primer (*e.g.*, oligonucleotide) typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, 75, 80, 85, 90, 95, or 100 or more consecutive nucleotides of a sense sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, of an anti-sense sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

Exemplary probes or primers are at least 12, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or more nucleotides in length and/or comprise consecutive nucleotides of an isolated nucleic acid molecule described herein. Probes based on the HST-1 nucleotide sequences can be used to detect (*e.g.*, specifically detect) transcripts or genomic sequences encoding the same or homologous polypeptides. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. In another embodiment a set of primers is provided, *e.g.*, primers suitable for use in a PCR, which can be used to amplify a selected region of an HST-1 sequence, *e.g.*, a domain, region, site or other sequence described herein. The primers should be at least 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or more nucleotides in length. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress an HST-1 polypeptide, such as by measuring a level of an HST-1-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting HST-1 mRNA levels or determining whether a genomic HST-1 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of an HST-1 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with

ATCC as Accession Number _____, which encodes a polypeptide having an HST-1 biological activity (the biological activities of the HST-1 polypeptides are described herein), expressing the encoded portion of the HST-1 polypeptide (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the HST-1 polypeptide. In an exemplary embodiment, the nucleic acid molecule is at least 50, 57, 63, 72, 100, 124, 150, 172, 175, 200, 250, 268, 300, 305, 328, 350, 400, 431, 450, 495, 500, 550, 600, 650, 700, 750, 800, 804, 850, 900, 950, 1000, 1050, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900 or more nucleotides in length and encodes a polypeptide having an HST-1 activity (as described herein).

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. Such differences can be due to due to degeneracy of the genetic code, thus resulting in a nucleic acid which encodes the same HST-1 polypeptides as those encoded by the nucleotide sequence shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a polypeptide having an amino acid sequence which differs by at least 1, but no greater than 5, 10, 20, 50, 100, 150, 155, 200, 250, 300, 350, 350, 400, 450, or 500 amino acid residues from the amino acid sequence shown in SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____. In yet another embodiment, the nucleic acid molecule encodes the amino acid sequence of human HST-1. If an alignment is needed for this comparison, the sequences should be aligned for maximum homology.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologues (different locus), and orthologues (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

Allelic variants result, for example, from DNA sequence polymorphisms within a population (*e.g.*, the human population) that lead to changes in the amino acid sequences of the HST-1 polypeptides. Such genetic polymorphism in the HST-1 genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding an HST-1 polypeptide, preferably a mammalian HST-1 polypeptide, and can further include non-coding regulatory sequences, and introns.

Accordingly, in one embodiment, the invention features isolated nucleic acid molecules which encode a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the nucleic acid molecule hybridizes to a complement of a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3, for example, under stringent hybridization conditions.

Allelic variants of human HST-1 include both functional and non-functional HST-1 polypeptides. Functional allelic variants are naturally occurring amino acid sequence variants of the human HST-1 polypeptide that have an HST-1 activity, *e.g.*, maintain the ability to bind an HST-1 ligand or substrate and/or modulate sugar transport, or sugar homeostasis. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, or substitution, deletion or insertion of non-critical residues in non-critical regions of the polypeptide.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human HST-1 polypeptide that do not have an HST-1 activity, *e.g.*, they do not have the ability to transport sugars into and out of cells or to modulate sugar homeostasis. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO:2, or a substitution, insertion or deletion in critical residues or critical regions.

The present invention further provides non-human orthologues of the human HST-1 polypeptide. Orthologues of human HST-1 polypeptides are polypeptides that are isolated from non-human organisms and possess the same HST-1 activity, *e.g.*, ligand binding and/or modulation of sugar transport mechanisms, as the human HST-1 polypeptide. Orthologues of the human HST-1 polypeptide can readily be identified as comprising an amino acid sequence that is substantially identical to SEQ ID NO:2.

Moreover, nucleic acid molecules encoding other HST-1 family members and, thus, which have a nucleotide sequence which differs from the HST-1 sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention. For example, another HST-1 cDNA can be identified based on the nucleotide sequence of human HST-1. Moreover, nucleic acid molecules encoding HST-1 polypeptides from different species, and which, thus, have a nucleotide sequence which differs from the HST-1 sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ are intended to be within the scope of the invention. For example, a mouse HST-1 cDNA can be identified based on the nucleotide sequence of a human HST-1.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the HST-1 cDNAs of the invention can be isolated based on their homology to the HST-1 nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. Nucleic acid molecules corresponding to natural allelic variants and homologues of the HST-1 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the HST-1 gene.

Orthologues, homologues and allelic variants can be identified using methods known in the art (*e.g.*, by hybridization to an isolated nucleic acid molecule of the present invention, for example, under stringent hybridization conditions). In one embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In other embodiment, the nucleic acid is at least 50, 57, 63, 72, 100, 124, 150, 172, 175, 200, 250, 268, 300, 305, 328, 350, 400, 431, 450, 495, 500, 550, 600, 650, 700, 750, 800, 804, 850, 900, 950, 1000, 1050, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900 or more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70° C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70° C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-

50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C) = 2(\#\text{ of A + T bases}) + 4(\#\text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1xSSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C, see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 or 3 and corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural polypeptide).

In addition to naturally-occurring allelic variants of the HST-1 sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, thereby leading to changes in the amino acid sequence of the encoded HST-1 polypeptides, without altering the functional ability of the HST-1 polypeptides. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of HST-1 (*e.g.*, the sequence of SEQ ID NO:2) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the HST-1 polypeptides of the present invention, *e.g.*,

those present in a transmembrane domain and/or a sugar transporter family domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the HST-1 polypeptides of the present invention and other members of the HST-1 family are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding HST-1 polypeptides that contain changes in amino acid residues that are not essential for activity. Such HST-1 polypeptides differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more identical to SEQ ID NO:2 (*e.g.*, to the entire length of SEQ ID NO:2).

An isolated nucleic acid molecule encoding an HST-1 polypeptide identical to the polypeptide of SEQ ID NO:2, can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded polypeptide. Mutations can be introduced into SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____ by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in an HST-1 polypeptide is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an HST-1 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for HST-1 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, the encoded polypeptide can be expressed recombinantly and the activity of the polypeptide can be determined.

In a preferred embodiment, a mutant HST-1 polypeptide can be assayed for the ability to (1) maintain sugar homeostasis in a cell, (2) influence insulin and/or glucagon secretion, (3) bind a monosaccharide, *e.g.*, D-glucose, D-fructose, and/or D-galactose, and (4) transport monosaccharides across a cell membrane.

In addition to the nucleic acid molecules encoding HST-1 polypeptides described above, another aspect of the invention pertains to isolated nucleic acid molecules which are antisense thereto. In an exemplary embodiment, the invention provides an isolated nucleic acid molecule which is antisense to an HST-1 nucleic acid molecule (*e.g.*, is antisense to the coding strand of an HST-1 nucleic acid molecule). An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a polypeptide, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire HST-1 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding HST-1. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the coding region of human HST-1 corresponds to SEQ ID NO:3). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding HST-1. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding HST-1 disclosed herein (*e.g.*, SEQ ID NO:3), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of HST-1 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of HST-1 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of HST-1 mRNA (*e.g.*, between the –10 and +10 regions of the start site of a gene nucleotide sequence). An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic

acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding an HST-1 polypeptide to thereby inhibit expression of the polypeptide, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide

(Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave HST-1 mRNA transcripts to thereby inhibit translation of HST-1 mRNA. A ribozyme having specificity for an HST-1-encoding nucleic acid can be designed based upon the nucleotide sequence of an HST-1 cDNA disclosed herein (*i.e.*, SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an HST-1-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, HST-1 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

Alternatively, HST-1 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the HST-1 (*e.g.*, the HST-1 promoter and/or enhancers) to form triple helical structures that prevent transcription of the HST-1 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15.

In yet another embodiment, the HST-1 nucleic acid molecules of the present invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al. Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of HST-1 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of HST-1 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In another embodiment, PNAs of HST-1 can be modified, (*e.g.*, to enhance their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of HST-1 nucleic acid molecules can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNase H and DNA polymerases), to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. (1996) *supra* and Finn P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

Alternatively, the expression characteristics of an endogenous HST-1 gene within a cell line or microorganism may be modified by inserting a heterologous DNA regulatory element into the genome of a stable cell line or cloned microorganism such that the inserted regulatory element is operatively linked with the endogenous HST-1 gene. For example, an endogenous HST-1 gene which is normally "transcriptionally silent", *i.e.*, an HST-1 gene which is normally not expressed, or is expressed only at very low levels in a cell line or microorganism, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell line or microorganism. Alternatively, a transcriptionally silent, endogenous HST-1 gene may be activated by insertion of a promiscuous regulatory element that works across cell types.

A heterologous regulatory element may be inserted into a stable cell line or cloned microorganism, such that it is operatively linked with an endogenous HST-1 gene, using techniques, such as targeted homologous recombination, which are well known to those of skill in the art, and described, *e.g.*, in Chappel, U.S. Patent No. 5,272,071; PCT publication No. WO 91/06667, published May 16, 1991.

II. Isolated HST-1 Polypeptides and Anti-HST-1 Antibodies

One aspect of the invention pertains to isolated HST-1 or recombinant polypeptides and polypeptides, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-HST-1 antibodies. In one embodiment, native HST-1 polypeptides can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, HST-1 polypeptides are produced by recombinant DNA techniques. Alternative to recombinant expression, an HST-1 polypeptide or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the HST-1 polypeptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of HST-1 polypeptide in which the polypeptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of HST-1 polypeptide having less than about 30% (by dry weight) of non-HST-1 polypeptide (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-HST-1 polypeptide, still more preferably less than about 10% of non-HST-1 polypeptide, and most preferably less than about 5% non-HST-1 polypeptide. When the HST-1 polypeptide or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*,

culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of HST-1 polypeptide in which the polypeptide is separated from chemical precursors or other chemicals which are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of HST-1 polypeptide having less than about 30% (by dry weight) of chemical precursors or non-HST-1 chemicals, more preferably less than about 20% chemical precursors or non-HST-1 chemicals, still more preferably less than about 10% chemical precursors or non-HST-1 chemicals, and most preferably less than about 5% chemical precursors or non-HST-1 chemicals.

As used herein, a "biologically active portion" of an HST-1 polypeptide includes a fragment of an HST-1 polypeptide which participates in an interaction between an HST-1 molecule and a non-HST-1 molecule. Biologically active portions of an HST-1 polypeptide include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the HST-1 polypeptide, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, which include less amino acids than the full length HST-1 polypeptides, and exhibit at least one activity of an HST-1 polypeptide. Typically, biologically active portions comprise a domain or motif with at least one activity of the HST-1 polypeptide, *e.g.*, modulating sugar transport mechanisms. A biologically active portion of an HST-1 polypeptide can be a polypeptide which is, for example, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 155, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550 or more amino acids in length. Biologically active portions of an HST-1 polypeptide can be used as targets for developing agents which modulate an HST-1 mediated activity, *e.g.*, a sugar transport mechanism.

In one embodiment, a biologically active portion of an HST-1 polypeptide comprises at least one transmembrane domain. It is to be understood that a preferred biologically active portion of an HST-1 polypeptide of the present invention comprises at least one or more of the following domains: a transmembrane domain and/or a sugar transporter family domain. Moreover, other biologically active portions, in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native HST-1 polypeptide.

Another aspect of the invention features fragments of the polypeptide having the amino acid sequence of SEQ ID NO:2, for example, for use as immunogens. In one embodiment, a fragment comprises at least 5 amino acids (*e.g.*, contiguous or consecutive amino acids) of the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____. In another embodiment, a fragment comprises at least 10, 15, 20, 25, 30, 35, 40, 45,

50 or more amino acids (*e.g.*, contiguous or consecutive amino acids) of the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number _____.

In a preferred embodiment, an HST-1 polypeptide has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the HST-1 polypeptide is substantially identical to SEQ ID NO:2, and retains the functional activity of the polypeptide of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection I above. In another embodiment, the HST-1 polypeptide is a polypeptide which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more identical to SEQ ID NO:2.

In another embodiment, the invention features an HST-1 polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more identical to a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or a complement thereof. This invention further features an HST-1 polypeptide which is encoded by a nucleic acid molecule consisting of a nucleotide sequence which hybridizes under stringent hybridization conditions to a complement of a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, or a complement thereof.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the HST-1 amino acid sequence of SEQ ID NO:2 having 419 amino acid residues, at least 126, preferably at least 168, more preferably at least 210, more preferably at least 251, even more preferably at least 293, and even more preferably at least 335 or 377 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions

shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction with the GAP program include a Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0 or version 2.0U), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and polypeptide sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to HST-1 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 100, wordlength = 3, and a Blosum62 matrix to obtain amino acid sequences homologous to HST-1 polypeptide molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The invention also provides HST-1 chimeric or fusion proteins. As used herein, an HST-1 "chimeric protein" or "fusion protein" comprises an HST-1 polypeptide operatively linked to a non-HST-1 polypeptide. An "HST-1 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to HST-1, whereas a "non-HST-1 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not

substantially homologous to the HST-1 polypeptide, *e.g.*, a polypeptide which is different from the HST-1 polypeptide and which is derived from the same or a different organism. Within an HST-1 fusion protein the HST-1 polypeptide can correspond to all or a portion of an HST-1 polypeptide. In a preferred embodiment, an HST-1 fusion protein comprises at least one biologically active portion of an HST-1 polypeptide. In another preferred embodiment, an HST-1 fusion protein comprises at least two biologically active portions of an HST-1 polypeptide. Within the fusion protein, the term "operatively linked" is intended to indicate that the HST-1 polypeptide and the non-HST-1 polypeptide are fused in-frame to each other. The non-HST-1 polypeptide can be fused to the N-terminus or C-terminus of the HST-1 polypeptide.

For example, in one embodiment, the fusion protein is a GST-HST-1 fusion protein in which the HST-1 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant HST-1.

In another embodiment, the fusion protein is an HST-1 polypeptide containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of HST-1 can be increased through the use of a heterologous signal sequence.

The HST-1 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The HST-1 fusion proteins can be used to affect the bioavailability of an HST-1 substrate. Use of HST-1 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding an HST-1 polypeptide; (ii) mis-regulation of the HST-1 gene; and (iii) aberrant post-translational modification of an HST-1 polypeptide.

Moreover, the HST-1-fusion proteins of the invention can be used as immunogens to produce anti-HST-1 antibodies in a subject, to purify HST-1 ligands and in screening assays to identify molecules which inhibit the interaction of HST-1 with an HST-1 substrate.

Preferably, an HST-1 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992).

Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An HST-1-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the HST-1 polypeptide.

The present invention also pertains to variants of the HST-1 polypeptides which function as either HST-1 agonists (mimetics) or as HST-1 antagonists. Variants of the HST-1 polypeptides can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of an HST-1 polypeptide. An agonist of the HST-1 polypeptides can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of an HST-1 polypeptide. An antagonist of an HST-1 polypeptide can inhibit one or more of the activities of the naturally occurring form of the HST-1 polypeptide by, for example, competitively modulating an HST-1-mediated activity of an HST-1 polypeptide. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the polypeptide has fewer side effects in a subject relative to treatment with the naturally occurring form of the HST-1 polypeptide.

In one embodiment, variants of an HST-1 polypeptide which function as either HST-1 agonists (mimetics) or as HST-1 antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of an HST-1 polypeptide for HST-1 polypeptide agonist or antagonist activity. In one embodiment, a variegated library of HST-1 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of HST-1 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential HST-1 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of HST-1 sequences therein. There are a variety of methods which can be used to produce libraries of potential HST-1 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential HST-1 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucleic Acid Res.* 11:477.

In addition, libraries of fragments of an HST-1 polypeptide coding sequence can be used to generate a variegated population of HST-1 fragments for screening and subsequent selection of variants of an HST-1 polypeptide. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an

HST-1 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the HST-1 polypeptide.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of HST-1 polypeptides. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify HST-1 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

In one embodiment, cell based assays can be exploited to analyze a variegated HST-1 library. For example, a library of expression vectors can be transfected into a cell line, *e.g.*, an endothelial cell line, which ordinarily responds to HST-1 in a particular HST-1 substrate-dependent manner. The transfected cells are then contacted with HST-1 and the effect of expression of the mutant on signaling by the HST-1 substrate can be detected, *e.g.*, by monitoring intracellular calcium, IP3, or diacylglycerol concentration, phosphorylation profile of intracellular proteins, or the activity of an HST-1-regulated transcription factor. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the HST-1 substrate, and the individual clones further characterized.

An isolated HST-1 polypeptide, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind HST-1 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length HST-1 polypeptide can be used or, alternatively, the invention provides antigenic peptide fragments of HST-1 for use as immunogens. The antigenic peptide of HST-1 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 and encompasses an epitope of HST-1 such that an antibody raised against the peptide forms a specific immune complex with HST-1. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more

preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of HST-1 that are located on the surface of the polypeptide, *e.g.*, hydrophilic regions, as well as regions with high antigenicity (see, for example, Figure 2).

An HST-1 immunogen typically is used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed HST-1 polypeptide or a chemically synthesized HST-1 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic HST-1 preparation induces a polyclonal anti-HST-1 antibody response.

Accordingly, another aspect of the invention pertains to anti-HST-1 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as HST-1. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind HST-1. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of HST-1. A monoclonal antibody composition thus typically displays a single binding affinity for a particular HST-1 polypeptide with which it immunoreacts.

Polyclonal anti-HST-1 antibodies can be prepared as described above by immunizing a suitable subject with an HST-1 immunogen. The anti-HST-1 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized HST-1. If desired, the antibody molecules directed against HST-1 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-HST-1 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem* .255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.*

(1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an HST-1 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds HST-1.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-HST-1 monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* *Somatic Cell Genet.*, cited *supra*; Lerner, *Yale J. Biol. Med.*, cited *supra*; Kenneth, *Monoclonal Antibodies*, cited *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind HST-1, *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-HST-1 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with HST-1 to thereby isolate immunoglobulin library members that bind HST-1. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display

library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al. Nature* (1990) 348:552-554.

Additionally, recombinant anti-HST-1 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-HST-1 antibody (*e.g.*, monoclonal antibody) can be used to isolate HST-1 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-HST-1 antibody can facilitate the purification of natural HST-1 from cells and of recombinantly produced HST-1 expressed in host cells. Moreover, an anti-HST-1 antibody can be used to detect HST-1 polypeptide (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the HST-1 polypeptide. Anti-HST-1 antibodies can be used diagnostically to monitor polypeptide levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given

treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, for example recombinant expression vectors, containing a nucleic acid containing an HST-1 nucleic acid molecule or vectors containing a nucleic acid molecule which encodes an HST-1 polypeptide (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably

linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, HST-1 polypeptides, mutant forms of HST-1 polypeptides, fusion proteins, and the like).

Accordingly, an exemplary embodiment provides a method for producing a polypeptide, preferably an HST-1 polypeptide, by culturing in a suitable medium a host cell of the invention (*e.g.*, a mammalian host cell such as a non-human mammalian cell) containing a recombinant expression vector, such that the polypeptide is produced.

The recombinant expression vectors of the invention can be designed for expression of HST-1 polypeptides in prokaryotic or eukaryotic cells. For example, HST-1 polypeptides can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical

fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in HST-1 activity assays, (*e.g.*, direct assays or competitive assays described in detail below), or to generate antibodies specific for HST-1 polypeptides, for example. In a preferred embodiment, an HST-1 fusion protein expressed in a retroviral expression vector of the present invention can be utilized to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (*e.g.*, six (6) weeks).

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the HST-1 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *Embo J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (InVitrogen Corp, San Diego, CA).

Alternatively, HST-1 polypeptides can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include the pAc series (Smith *et al.* (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to HST-1 mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et*

al., Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which an HST-1 nucleic acid molecule of the invention is introduced, *e.g.*, an HST-1 nucleic acid molecule within a vector (*e.g.*, a recombinant expression vector) or an HST-1 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, an HST-1 polypeptide can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding an HST-1 polypeptide or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an HST-1 polypeptide. Accordingly, the invention further provides methods for producing an HST-1 polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding an HST-1 polypeptide has been introduced) in a suitable medium such that an HST-1 polypeptide is produced. In another embodiment, the method further comprises isolating an HST-1 polypeptide from the medium or the host cell.

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which HST-1-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous HST-1 sequences have been introduced into their genome or homologous recombinant animals in which endogenous HST-1 sequences have been altered. Such animals are useful for studying the function and/or activity of an HST-1 and for identifying and/or evaluating modulators of HST-1 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous HST-1 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing an HST-1-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The HST-1 cDNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human HST-1 gene, such as a mouse or rat HST-1 gene, can be used as a transgene. Alternatively, an HST-1 gene homologue, such as another HST-1 family member, can be isolated based on hybridization to the HST-1 cDNA sequences of SEQ ID NO:1 or 3, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____ (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A

tissue-specific regulatory sequence(s) can be operably linked to an HST-1 transgene to direct expression of an HST-1 polypeptide to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of an HST-1 transgene in its genome and/or expression of HST-1 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding an HST-1 polypeptide can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an HST-1 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the HST-1 gene. The HST-1 gene can be a human gene (*e.g.*, the cDNA of SEQ ID NO:3), but more preferably, is a non-human homologue of a human HST-1 gene (*e.g.*, a cDNA isolated by stringent hybridization with the nucleotide sequence of SEQ ID NO:1). For example, a mouse HST-1 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous HST-1 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous HST-1 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous HST-1 gene is mutated or otherwise altered but still encodes functional polypeptide (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous HST-1 polypeptide). In the homologous recombination nucleic acid molecule, the altered portion of the HST-1 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the HST-1 gene to allow for homologous recombination to occur between the exogenous HST-1 gene carried by the homologous recombination nucleic acid molecule and an endogenous HST-1 gene in a cell, *e.g.*, an embryonic stem cell. The additional flanking HST-1 nucleic acid sequence is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for a description of homologous recombination vectors). The homologous recombination nucleic acid molecule is introduced into a cell, *e.g.*, an

embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced HST-1 gene has homologously recombined with the endogenous HST-1 gene are selected (see *e.g.*, Li, E. *et al.* (1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, *e.g.*, Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

IV. Pharmaceutical Compositions

The HST-1 nucleic acid molecules, fragments of HST-1 polypeptides, and anti-HST-1 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, polypeptide, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the

use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a fragment of an HST-1 polypeptide or an anti-HST-1 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal

sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form

employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a polypeptide or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*,. including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which

the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-

interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, an HST-1 polypeptide of the invention has one or more of the following activities: (1) maintain sugar homeostasis in a cell, (2) influence insulin and/or glucagon

secretion, (3) bind a monosaccharide, *e.g.*, D-glucose, D-fructose, and/or D-galactose, and (4) transport monosaccharides across a cell membrane.

The isolated nucleic acid molecules of the invention can be used, for example, to express HST-1 polypeptides (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect HST-1 mRNA (*e.g.*, in a biological sample) or a genetic alteration in an HST-1 gene, and to modulate HST-1 activity, as described further below. The HST-1 polypeptides can be used to treat disorders characterized by insufficient or excessive production of an HST-1 substrate or production of HST-1 inhibitors. In addition, the HST-1 polypeptides can be used to screen for naturally occurring HST-1 substrates, to screen for drugs or compounds which modulate HST-1 activity, as well as to treat disorders characterized by insufficient or excessive production of HST-1 polypeptide or production of HST-1 polypeptide forms which have decreased, aberrant or unwanted activity compared to HST-1 wild type polypeptide (*e.g.*, sugar transporter disorders). Moreover, the anti-HST-1 antibodies of the invention can be used to detect and isolate HST-1 polypeptides, to regulate the bioavailability of HST-1 polypeptides, and modulate HST-1 activity.

A. Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) which bind to HST-1 polypeptides, have a stimulatory or inhibitory effect on, for example, HST-1 expression or HST-1 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of HST-1 substrate.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of an HST-1 polypeptide or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of an HST-1 polypeptide or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses an HST-1 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate HST-1 activity is determined. Determining the ability of the test compound to modulate HST-1 activity can be accomplished by monitoring, for example, intracellular or extracellular D-glucose, D-fructose or D-galactose concentration, or insulin or glucagon secretion. The cell, for example, can be of mammalian origin, *e.g.*, a liver cell, fat cell, muscle cell, or a blood cell, such as an erythrocyte.

The ability of the test compound to modulate HST-1 binding to a substrate or to bind to HST-1 can also be determined. Determining the ability of the test compound to modulate HST-1 binding to a substrate can be accomplished, for example, by coupling the HST-1 substrate with a radioisotope or enzymatic label such that binding of the HST-1 substrate to HST-1 can be determined by detecting the labeled HST-1 substrate in a complex. Alternatively, HST-1 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate HST-1 binding to an HST-1 substrate in a complex. Determining the ability of the test compound to bind HST-1 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to HST-1 can be determined by detecting the labeled HST-1 compound in a complex. For example, compounds (*e.g.*, HST-1 substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (*e.g.*, an HST-1 substrate) to interact with HST-1 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with HST-1 without the labeling of either the compound or the HST-1. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and HST-1.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing an HST-1 target molecule (*e.g.*, an HST-1 substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the HST-1 target molecule. Determining the ability of the test compound to modulate the activity of an HST-1 target molecule can be accomplished, for example, by determining the ability of the HST-1 polypeptide to bind to or interact with the HST-1 target molecule.

Determining the ability of the HST-1 polypeptide, or a biologically active fragment thereof, to bind to or interact with an HST-1 target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the HST-1 polypeptide to bind to or interact with an HST-1 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular Ca^{2+}, diacylglycerol, IP_3, and the like), detecting catalytic/enzymatic activity of the target using an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response.

In yet another embodiment, an assay of the present invention is a cell-free assay in which an HST-1 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the HST-1 polypeptide or biologically active portion thereof is determined. Preferred biologically active portions of the HST-1 polypeptides to be used in assays of the present invention include fragments which participate in interactions with non-HST-1 molecules, *e.g.*, fragments with high surface probability scores (see, for example, Figure 2). Binding of the test compound to the HST-1 polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the HST-1 polypeptide or biologically active portion thereof with a known compound which binds HST-1 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of

the test compound to interact with an HST-1 polypeptide, wherein determining the ability of the test compound to interact with an HST-1 polypeptide comprises determining the ability of the test compound to preferentially bind to HST-1 or biologically active portion thereof as compared to the known compound.

In another embodiment, the assay is a cell-free assay in which an HST-1 polypeptide or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the HST-1 polypeptide or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of an HST-1 polypeptide can be accomplished, for example, by determining the ability of the HST-1 polypeptide to bind to an HST-1 target molecule by one of the methods described above for determining direct binding. Determining the ability of the HST-1 polypeptide to bind to an HST-1 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705. As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In an alternative embodiment, determining the ability of the test compound to modulate the activity of an HST-1 polypeptide can be accomplished by determining the ability of the HST-1 polypeptide to further modulate the activity of a downstream effector of an HST-1 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting an HST-1 polypeptide or biologically active portion thereof with a known compound which binds the HST-1 polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the HST-1 polypeptide, wherein determining the ability of the test compound to interact with the HST-1 polypeptide comprises determining the ability of the HST-1 polypeptide to preferentially bind to or modulate the activity of an HST-1 target molecule.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either HST-1 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to an HST-1 polypeptide, or interaction of an HST-1 polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for

containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/ HST-1 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized micrometer plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or HST-1 polypeptide, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or micrometer plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of HST-1 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either an HST-1 polypeptide or an HST-1 target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated HST-1 polypeptide or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with HST-1 polypeptide or target molecules but which do not interfere with binding of the HST-1 polypeptide to its target molecule can be derivatized to the wells of the plate, and unbound target or HST-1 polypeptide trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the HST-1 polypeptide or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the HST-1 polypeptide or target molecule.

In another embodiment, modulators of HST-1 expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of HST-1 mRNA or polypeptide in the cell is determined. The level of expression of HST-1 mRNA or polypeptide in the presence of the candidate compound is compared to the level of expression of HST-1 mRNA or polypeptide in the absence of the candidate compound. The candidate compound can then be identified as a modulator of HST-1 expression based on this comparison. For example, when expression of HST-1 mRNA or polypeptide is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of HST-1 mRNA or polypeptide expression. Alternatively, when expression of HST-1 mRNA or polypeptide is less (statistically significantly less) in the presence of the candidate compound than in its

absence, the candidate compound is identified as an inhibitor of HST-1 mRNA or polypeptide expression. The level of HST-1 mRNA or polypeptide expression in the cells can be determined by methods described herein for detecting HST-1 mRNA or polypeptide.

In yet another aspect of the invention, the HST-1 polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with HST-1 ("HST-1-binding proteins" or "HST-1-bp") and are involved in HST-1 activity. Such HST-1-binding proteins are also likely to be involved in the propagation of signals by the HST-1 polypeptides or HST-1 targets as, for example, downstream elements of an HST-1-mediated signaling pathway. Alternatively, such HST-1-binding proteins are likely to be HST-1 inhibitors.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for an HST-1 polypeptide is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo,* forming an HST-1-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the HST-1 polypeptide.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of an HST-1 polypeptide can be confirmed *in vivo*, *e.g.*, in an animal such as an animal model for obesity, or diabetes. Examples of animals that can be used include the transgenic mouse described in U.S. Patent No. 5,932,779 that contains a mutation in an endogenous melanocortin-4-receptor (MC4-R) gene; animals having mutations which lead to syndromes that include obesity symptoms (described in, for example, Friedman, J. M. *et al.* (1991) *Mamm. Gen.* 1:130-144; Friedman, J. M. and Liebel, R. L. (1992) *Cell* 69:217-220; Bray, G. A. (1992) *Prog. Brain Res.* 93:333-341; and Bray, G. A. (1989) *Amer. J. Clin. Nutr.* 5:891-902); the animals described in Stubdal H. *et al.* (2000) *Mol. Cell Biol.* 20(3):878-82 (the mouse tubby

phenotype characterized by maturity-onset obesity); the animals described in Abadie J.M. *et al. Lipids* (2000) 35(6):613-20 (the obese Zucker rat (ZR), a genetic model of human youth-onset obesity and type 2 diabetes mellitus); the animals described in Shaughnessy S. *et al.* (2000) *Diabetes* 49(6):904-11 (mice null for the adipocyte fatty acid binding protein); or the animals described in Loskutoff D.J. *et al.* (2000) *Ann. N. Y. Acad. Sci.* 902:272-81 (the fat mouse). Other examples of animals that may be used include non-recombinant, non-genetic animal models of obesity such as, for example, rabbit, mouse, or rat models in which the animal has been exposed to either prolonged cold or long-term over-eating, thereby, inducing hypertrophy of BAT and increasing BAT thermogenesis (Himms-Hagen, J. (1990), *supra)*. Additionally, animals created by ablation of BAT through use of targeted expression of a toxin gene (Lowell, B. *et al.* (1993) *Nature* 366:740-742) may be used.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, an HST-1 modulating agent, an antisense HST-1 nucleic acid molecule, an HST-1-specific antibody, or an HST-1-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. This process is called chromosome mapping. Accordingly, portions or fragments of the HST-1 nucleotide sequences, described herein, can be used to map the location of the HST-1 genes on a chromosome. The mapping of the HST-1 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, HST-1 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the HST-1 nucleotide sequences. Computer analysis of the HST-1 sequences can be used to predict primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the HST-1 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow, because they lack a particular enzyme, but human cells can, the one human chromosome that contains the gene encoding the needed enzyme, will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the HST-1 nucleotide sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map an HST-1 sequence to its chromosome include *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical such as colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good

results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature,* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the HST-1 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The HST-1 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the HST-1 nucleotide sequences described herein

can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The HST-1 nucleotide sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from HST-1 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of HST-1 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated

fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the HST-1 nucleotide sequences or portions thereof, *e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases.

The HST-1 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such HST-1 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, HST-1 primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

C. Predictive Medicine:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining HST-1 polypeptide and/or nucleic acid expression as well as HST-1 activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant or unwanted HST-1 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with HST-1 polypeptide, nucleic acid expression or activity. For example, mutations in an HST-1 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with HST-1 polypeptide, nucleic acid expression or activity.

Another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of HST-1 in clinical trials.

These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of HST-1 polypeptide or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting HST-1 polypeptide or nucleic acid (*e.g.*, mRNA, or genomic DNA) that encodes HST-1 polypeptide such that the presence of HST-1 polypeptide or nucleic acid is detected in the biological sample. In another aspect, the present invention provides a method for detecting the presence of HST-1 activity in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of HST-1 activity such that the presence of HST-1 activity is detected in the biological sample. A preferred agent for detecting HST-1 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to HST-1 mRNA or genomic DNA. The nucleic acid probe can be, for example, the HST-1 nucleic acid set forth in SEQ ID NO:1 or 3, or the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to HST-1 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting HST-1 polypeptide is an antibody capable of binding to HST-1 polypeptide, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect HST-1 mRNA, polypeptide, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of HST-1 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of HST-1 polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of HST-1 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of HST-1 polypeptide include introducing into a subject a labeled anti-HST-1 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

The present invention also provides diagnostic assays for identifying the presence or absence of a genetic alteration characterized by at least one of (i) aberrant modification or mutation of a gene encoding an HST-1 polypeptide; (ii) aberrant expression of a gene encoding an HST-1 polypeptide; (iii) mis-regulation of the gene; and (iii) aberrant post-translational modification of an HST-1 polypeptide, wherein a wild-type form of the gene encodes a polypeptide with an HST-1 activity. "Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes, but is not limited to, expression at non-wild type levels (*e.g.*, over or under expression); a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed (*e.g.*, increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage); a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-transitional modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene (*e.g.*, a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus).

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a serum sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting HST-1 polypeptide, mRNA, or genomic DNA, such that the presence of HST-1 polypeptide, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of HST-1 polypeptide, mRNA or genomic DNA in the control sample with the presence of HST-1 polypeptide, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of HST-1 in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting HST-1 polypeptide or mRNA in a biological sample; means for determining the amount of HST-1 in the sample; and means for comparing the amount of HST-1 in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect HST-1 polypeptide or nucleic acid.

2. Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant or unwanted HST-1 expression or activity. As used herein, the term "aberrant" includes an HST-1 expression or activity which deviates from the wild type HST-1 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant HST-1 expression or activity is intended to include the cases in which a mutation in the HST-1 gene causes the HST-1 gene to be under-expressed or over-expressed and situations in which such mutations result in a non-functional HST-1 polypeptide or a polypeptide which does not function in a wild-type fashion, *e.g.*, a polypeptide which does not interact with an HST-1 substrate, *e.g.*, a sugar transporter subunit or ligand, or one which interacts with a non-HST-1 substrate, *e.g.* a non-sugar transporter subunit or ligand. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response, such as cellular proliferation. For example, the term unwanted includes an HST-1 expression or activity which is undesirable in a subject.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with a misregulation in HST-1 polypeptide activity or nucleic acid expression, such as a sugar transporter disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disorder associated with a misregulation in HST-1 polypeptide activity or nucleic acid expression, such as a sugar transporter disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant or unwanted HST-1 expression or activity in which a test sample is obtained from a subject and HST-1 polypeptide or nucleic acid (*e.g.*, mRNA or genomic DNA) is detected, wherein the presence of HST-1 polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted HST-1 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted HST-1 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a sugar transporter disorder. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder

associated with aberrant or unwanted HST-1 expression or activity in which a test sample is obtained and HST-1 polypeptide or nucleic acid expression or activity is detected (*e.g.*, wherein the abundance of HST-1 polypeptide or nucleic acid expression or activity is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant or unwanted HST-1 expression or activity).

The methods of the invention can also be used to detect genetic alterations in an HST-1 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in HST-1 polypeptide activity or nucleic acid expression, such as a sugar transporter disorder, a sugar homeostasis disorder, or a disorder of cellular growth, differentiation, or migration. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding an HST-1 -polypeptide, or the mis-expression of the HST-1 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from an HST-1 gene; 2) an addition of one or more nucleotides to an HST-1 gene; 3) a substitution of one or more nucleotides of an HST-1 gene, 4) a chromosomal rearrangement of an HST-1 gene; 5) an alteration in the level of a messenger RNA transcript of an HST-1 gene, 6) aberrant modification of an HST-1 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of an HST-1 gene, 8) a non-wild type level of an HST-1-polypeptide, 9) allelic loss of an HST-1 gene, and 10) inappropriate post-translational modification of an HST-1-polypeptide. As described herein, there are a large number of assays known in the art which can be used for detecting alterations in an HST-1 gene. A preferred biological sample is a tissue or serum sample isolated by conventional means from a subject.

In certain embodiments, detection of the alteration involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the HST-1-gene (see Abravaya *et al.* (1995) *Nucleic Acids Res* .23:675-682). This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to an HST-1 gene under conditions such that hybridization and amplification of the HST-1-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary

amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in an HST-1 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in HST-1 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in HST-1 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra.* Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the HST-1 gene and detect mutations by comparing the sequence of the sample HST-1 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxam and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques*

19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No. WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the HST-1 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type HST-1 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in HST-1 cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on an HST-1 sequence, *e.g.*, a wild-type HST-1 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in HST-1 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control HST-1 nucleic acids will be denatured and

allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an HST-1 gene.

Furthermore, any cell type or tissue in which HST-1 is expressed may be utilized in the prognostic assays described herein.

3. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of an HST-1 polypeptide (*e.g.*, the modulation of sugar transport) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase HST-1 gene expression, polypeptide levels, or upregulate HST-1 activity, can be monitored in clinical trials of subjects exhibiting decreased HST-1 gene expression, polypeptide levels, or downregulated HST-1 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease HST-1 gene expression, polypeptide levels, or downregulate HST-1 activity, can be monitored in clinical trials of subjects exhibiting increased HST-1 gene expression, polypeptide levels, or upregulated HST-1 activity. In such clinical trials, the expression or activity of an HST-1 gene, and preferably, other genes that have been implicated in, for example, an HST-1-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

For example, and not by way of limitation, genes, including HST-1, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) which modulates HST-1 activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on HST-1-associated disorders (*e.g.*, disorders characterized by deregulated signaling or sugar transport), for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of HST-1 and other genes implicated in the HST-1-associated disorder, respectively. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of polypeptide produced, by one of the methods as described herein, or by measuring the levels of activity of HST-1 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of an HST-1 polypeptide, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the HST-1 polypeptide, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the HST-1 polypeptide, mRNA, or genomic DNA in the pre-administration sample with the HST-1 polypeptide, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of HST-1 to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of HST-1 to lower levels than detected, *i.e.* to decrease the effectiveness of the agent. According to such an embodiment, HST-1 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of an observable phenotypic response.

4. Electronic Apparatus Readable Media and Arrays

Electronic apparatus readable media comprising HST-1 sequence information is also provided. As used herein, "HST-1 sequence information" refers to any nucleotide and/or amino acid sequence information particular to the HST-1 molecules of the present invention, including but not limited to full-length nucleotide and/or amino acid sequences, partial nucleotide and/or amino acid sequences, polymorphic sequences including single nucleotide polymorphisms (SNPs), epitope sequences, and the like. Moreover, information "related to" said HST-1 sequence information includes detection of the presence or absence of a sequence (*e.g.*, detection of expression of a sequence, fragment, polymorphism, etc.), determination of the level of a sequence (*e.g.*, detection of a level of expression, for example, a quantitative detection), detection of a reactivity to a sequence (*e.g.*, detection of protein expression and/or levels, for example, using a sequence-specific antibody), and the like. As used herein, "electronic apparatus readable media" refers to any suitable medium for storing, holding or containing data or information that can be read and accessed directly by an electronic apparatus. Such media can include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc; electronic storage media such as RAM, ROM, EPROM, EEPROM and the like; general hard disks and hybrids of these categories such as

magnetic/optical storage media. The medium is adapted or configured for having recorded thereon HST-1 sequence information of the present invention.

As used herein, the term "electronic apparatus" is intended to include any suitable computing or processing apparatus or other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as a personal digital assistants (PDAs), cellular phone, pager and the like; and local and distributed processing systems.

As used herein, "recorded" refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the HST-1 sequence information.

A variety of software programs and formats can be used to store the sequence information on the electronic apparatus readable medium. For example, the sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like, as well as in other forms. Any number of dataprocessor structuring formats (*e.g.*, text file or database) may be employed in order to obtain or create a medium having recorded thereon the HST-1 sequence information.

By providing HST-1 sequence information in readable form, one can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the sequence information in readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

The present invention therefore provides a medium for holding instructions for performing a method for determining whether a subject has a HST-1-associated disease or disorder or a pre-disposition to a HST-1-associated disease or disorder, wherein the method comprises the steps of determining HST-1 sequence information associated with the subject and based on the HST-1 sequence information, determining whether the subject has a HST-1-associated disease or disorder or a pre-disposition to a HST-1-associated disease or disorder and/or recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a HST-1-associated disease or disorder or a pre-disposition to a disease associated with a HST-1 wherein the method comprises the steps

of determining HST-1 sequence information associated with the subject, and based on the HST-1 sequence information, determining whether the subject has a HST-1-associated disease or disorder or a pre-disposition to a HST-1-associated disease or disorder, and/or recommending a particular treatment for the disease, disorder or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

The present invention also provides in a network, a method for determining whether a subject has a HST-1-associated disease or disorder or a pre-disposition to a HST-1-associated disease or disorder associated with HST-1, said method comprising the steps of receiving HST-1 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to HST-1 and/or a HST-1-associated disease or disorder, and based on one or more of the phenotypic information, the HST-1 information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a HST-1-associated disease or disorder or a pre-disposition to a HST-1-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The present invention also provides a business method for determining whether a subject has a HST-1-associated disease or disorder or a pre-disposition to a HST-1-associated disease or disorder, said method comprising the steps of receiving information related to HST-1 (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information associated with the subject, acquiring information from the network related to HST-1 and/or related to a HST-1-associated disease or disorder, and based on one or more of the phenotypic information, the HST-1 information, and the acquired information, determining whether the subject has a HST-1-associated disease or disorder or a pre-disposition to a HST-1-associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder or pre-disease condition.

The invention also includes an array comprising a HST-1 sequence of the present invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be HST-1. This allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

In addition to such qualitative determination, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of

their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a HST-1-associated disease or disorder, progression of HST-1-associated disease or disorder, and processes, such a cellular transformation associated with the HST-1-associated disease or disorder.

The array is also useful for ascertaining the effect of the expression of a gene on the expression of other genes in the same cell or in different cells (*e.g.*, ascertaining the effect of HST-1 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

The array is also useful for ascertaining differential expression patterns of one or more genes in normal and abnormal cells. This provides a battery of genes (*e.g.*, including HST-1) that could serve as a molecular target for diagnosis or therapeutic intervention.

D. Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted HST-1 expression or activity, *e.g.* a sugar transporter disorder. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic

treatment with either the HST-1 molecules of the present invention or HST-1 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

Treatment is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted HST-1 expression or activity, by administering to the subject an HST-1 or an agent which modulates HST-1 expression or at least one HST-1 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted HST-1 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the HST-1 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of HST-1 aberrancy, for example, an HST-1, HST-1 agonist or HST-1 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating HST-1 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell capable of expressing HST-1 with an agent that modulates one or more of the activities of HST-1 polypeptide activity associated with the cell, such that HST-1 activity in the cell is modulated. An agent that modulates HST-1 polypeptide activity can be an agent as described herein, such as a nucleic acid or a polypeptide, a naturally-occurring target molecule of an HST-1 polypeptide (*e.g.*, an HST-1 substrate), an HST-1 antibody, an HST-1 agonist or antagonist, a peptidomimetic of an HST-1 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more HST-1 activities. Examples of such stimulatory agents include active HST-1 polypeptide and a nucleic acid molecule encoding HST-1 that has been

introduced into the cell. In another embodiment, the agent inhibits one or more HST-1 activities. Examples of such inhibitory agents include antisense HST-1 nucleic acid molecules, anti-HST-1 antibodies, and HST-1 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of an HST-1 polypeptide or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) HST-1 expression or activity. In another embodiment, the method involves administering an HST-1 polypeptide or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted HST-1 expression or activity.

Stimulation of HST-1 activity is desirable in situations in which HST-1 is abnormally downregulated and/or in which increased HST-1 activity is likely to have a beneficial effect. Likewise, inhibition of HST-1 activity is desirable in situations in which HST-1 is abnormally upregulated and/or in which decreased HST-1 activity is likely to have a beneficial effect.

3. Pharmacogenomics

The HST-1 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on HST-1 activity (*e.g.*, HST-1 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) HST-1-associated disorders (*e.g.*, proliferative disorders) associated with aberrant or unwanted HST-1 activity. In conjunction with such treatment, pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer an HST-1 molecule or HST-1 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with an HST-1 molecule or HST-1 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic

conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drugs target is known (*e.g.*, an HST-1 polypeptide of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have

been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, an HST-1 molecule or HST-1 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an HST-1 molecule or HST-1 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

4. Use of HST-1 Molecules as Surrogate Markers

The HST-1 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity of the HST-1 molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the HST-1 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a "surrogate marker" is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (*e.g.*, early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (*e.g.*, an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an

analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The HST-1 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a "pharmacodynamic marker" is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (*e.g.*, an HST-1 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-HST-1 antibodies may be employed in an immune-based detection system for an HST-1 polypeptide marker, or HST-1-specific radiolabeled probes may be used to detect an HST-1 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am, J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The HST-1 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, *e.g.*, McLeod *et al.* (1999) *Eur. J. Cancer* 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success,

may be selected. For example, based on the presence or quantity of RNA, or polypeptide (*e.g.*, HST-1 polypeptide or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in HST-1 DNA may correlate HST-1 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and the Sequence Listing, are incorporated herein by reference.

EXAMPLES

EXAMPLE 1: IDENTIFICATION AND CHARACTERIZATION OF HUMAN HST-1 cDNA

In this example, the identification and characterization of the gene encoding human HST-1 (clone 57250) is described.

Isolation of the human HST-1 cDNA

The invention is based, at least in part, on the discovery of a human gene encoding a novel polypeptide, referred to herein as human HST-1. The entire sequence of the human clone 57250 was determined and found to contain an open reading frame termed human "HST-1." The nucleotide sequence of the human HST-1 gene is set forth in Figure 1 and in the Sequence Listing as SEQ ID NO:1. The amino acid sequence of the human HST-1 expression product is set forth in Figures 1 and in the Sequence Listing as SEQ ID NO: 2. The HST-1 polypeptide comprises 572 amino acids. The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3. Clone 57250, comprising the coding region of human HST-1, was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.

Analysis of the Human HST-1 Molecules

The human HST-1 amino acid sequence was aligned with the amino acid sequence of the potent brain type organic ion transporter from *Homo sapiens* (Accession No. AB040056) using the CLUSTAL W (1.74) multiple sequence alignment program. The results of the alignment are set forth in Figure 5.

A search using the polypeptide sequence of SEQ ID NO:2 was performed against the HMM database in PFAM (Figure 3) resulting in the identification of a sugar transporter family domain in the amino acid sequence of human HST-1 at about residues 117-536 of SEQ ID NO:2, a potential UL25 domain in the amino acid sequence of human HST-1 at about residues 577-597 of SEQ ID NO:2 (score = 3.0), and a potential sodium: galactoside symporter family domain in the amino acid sequence of human HST-1 at about residues 287-541 of SEQ ID NO:2.

The amino acid sequence of human HST-1 was analyzed using the program PSORT (see the PSORT website) to predict the localization of the proteins within the cell. This program assesses the presence of different targeting and localization amino acid sequences within the query sequence. The results of this analysis indicated that human HST-1 may be localized to the endoplasmic reticulum, nucleus, secretory vesicles or mitochondria.

Searches of the amino acid sequence of human HST-1 were further performed against the Prosite database. These searches resulted in the identification in the amino acid sequence of human HST-1 of a potential N-glycosylation site, a number of potential protein kinase C phosphorylation sites, a number of potential casein kinase II phosphorylation sites, a number of potential N-myristoylation sites, a number of potential amidation sites, a potential prokaryotic membrane lipoprotein lipid attachment site, and a number of potential leucine zipper motifs.

A MEMSAT analysis of the polypeptide sequence of SEQ ID NO:2 was also performed (Figure 4), predicting twelve transmembrane domains in the amino acid sequence of human HST-1 (SEQ ID NO:2) at about residues 20-36, 150-167, 174-196, 204-220, 231-255, 263-282, 355-372, 387-405, 413-431, 438-462, 469-485, and 505-521.

Further domain motifs were identified by using the amino acid sequence of HST-1 (SEQ ID NO:2) to search through the ProDom database. Numerous matches against protein domains described as "transporter organic cation MBOCT potent brain type", "transporter organic cation anion transmembrane glycoprotein monoamine", "DNA packaging" and the like were identified.

EXAMPLE 2: EXPRESSION OF RECOMBINANT HST-1 POLYPEPTIDE IN BACTERIAL CELLS

In this example, human HST-1 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, HST-1 is fused to GST and this fusion polypeptide is expressed in *E. coli*, *e.g.*, strain PEB199. Expression of the GST-HST-1 fusion polypeptide in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione

beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

EXAMPLE 3: EXPRESSION OF RECOMBINANT HST-1 POLYPEPTIDE IN COS CELLS

To express the human HST-1 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire HST-1 polypeptide and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant polypeptide under the control of the CMV promoter.

To construct the plasmid, the human HST-1 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the HST-1 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the HST-1 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the HST-1 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5α, SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the human HST-1-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the IC54420 polypeptide is detected by radiolabelling (^{35}S-methionine or ^{35}S-cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are

labeled for 8 hours with ^{35}S-methionine (or ^{35}S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the human HST-1 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the HST-1 polypeptide is detected by radiolabelling and immunoprecipitation using an HST-1-specific monoclonal antibody.

EXAMPLE 4: TISSUE DISTRIBUTION OF HUMAN HST-1 mRNA USING TAQMAN™ ANALYSIS

This example describes the tissue distribution of human HST-1 mRNA in a variety of cells and tissues, as determined using the TaqMan™ procedure. The Taqman™ procedure is a quantitative, reverse transcription PCR-based approach for detecting mRNA. The RT-PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold™ DNA Polymerase to cleave a TaqMan™ probe during PCR. Briefly, cDNA was generated from the samples of interest, *e.g.*, various human tissue samples, and used as the starting material for PCR amplification. In addition to the 5' and 3' gene-specific primers, a gene-specific oligonucleotide probe (complementary to the region being amplified) was included in the reaction (*i.e.*, the Taqman™ probe). The TaqMan™ probe includes the oligonucleotide with a fluorescent reporter dye covalently linked to the 5' end of the probe (such as FAM (6-carboxyfluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein), or VIC) and a quencher dye (TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine) at the 3' end of the probe.

During the PCR reaction, cleavage of the probe separates the reporter dye and the quencher dye, resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'-3' nucleolytic activity of the AmpliTaq™ Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues. The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. RNA

was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no detectable PCR amplification of the control gene confirms efficient removal of genomic DNA contamination.

As indicated in Figure 6, strong expression of HST-1 was detected in human coronary smooth muscle cells and neutrophils, as well as in normal human pancreatic tissue and human lung tissue derived from normal, tumor, and chronic obstructive pulmonary disease samples. In addition, HST-1 expression was detected at moderate levels in normal ovary and lymph node tissues, breast tumor tissue, prostate tumor tissue, and in bone marrow mononuclear cells.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1. An isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:1; and

(b) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO:3.

2. An isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2.

3. An isolated nucleic acid molecule comprising the nucleotide sequence contained in the plasmid deposited with ATCC® as Accession Number _____.

4. An isolated nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2.

5. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 100 nucleotides of a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3, or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence at least about 60% identical to the amino acid sequence of SEQ ID NO:2; and

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 10 contiguous amino acid residues of the amino acid sequence of SEQ ID NO:2.

6. An isolated nucleic acid molecule which hybridizes to a complement of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 under stringent conditions.

7. An isolated nucleic acid molecule comprising a nucleotide sequence which is complementary to the nucleotide sequence of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.

8. An isolated nucleic acid molecule comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5, and a nucleotide sequence encoding a heterologous polypeptide.

9. A vector comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5.

10. The vector of claim 9, which is an expression vector.

11. A host cell transfected with the expression vector of claim 10.

12. A method of producing a polypeptide comprising culturing the host cell of claim 11 in an appropriate culture medium to, thereby, produce the polypeptide.

13. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the fragment comprises at least 10 contiguous amino acids of SEQ ID NO:2;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 or 3 under stringent conditions;

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 or 3; and

d) a polypeptide comprising an amino acid sequence which is at least 60% identical to the amino acid sequence of SEQ ID NO:2.

14. The isolated polypeptide of claim 13 comprising the amino acid sequence of SEQ ID NO:2.

15. The polypeptide of claim 13, further comprising heterologous amino acid sequences.

16. An antibody which selectively binds to a polypeptide of claim 13.

17. A method for detecting the presence of a polypeptide of claim 13 in a sample comprising:

a) contacting the sample with a compound which selectively binds to the polypeptide; and

b) determining whether the compound binds to the polypeptide in the sample to thereby detect the presence of a polypeptide of claim 13 in the sample.

18. The method of claim 17, wherein the compound which binds to the polypeptide is an antibody.

19. A kit comprising a compound which selectively binds to a polypeptide of claim 13 and instructions for use.

20. A method for detecting the presence of a nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in a sample comprising:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to a complement of the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to the complement of the nucleic acid molecule in the sample to thereby detect the presence of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 in the sample.

21. The method of claim 20, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

22. A kit comprising a compound which selectively hybridizes to a complement of the nucleic acid molecule of any one of claims 1, 2, 3, 4, or 5 and instructions for use.

23. A method for identifying a compound which binds to a polypeptide of claim 13 comprising:

a) contacting the polypeptide, or a cell expressing the polypeptide with a test compound; and

b) determining whether the polypeptide binds to the test compound.

24. The method of claim 23, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detection of test compound/polypeptide binding;

b) detection of binding using a competition binding assay; and

c) detection of binding using an assay for HST-1 activity.

25. A method for modulating the activity of a polypeptide of claim 13 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

26. A method for identifying a compound which modulates the activity of a polypeptide of claim 13 comprising:

a) contacting a polypeptide of claim 13 with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

Input file Fbh57250pat.seq; Output File Fbh57250pat.tra
Sequence length 1917

```
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 A  R  R  L  L  A  S  A  S  W  V  P  C  I  V  L  G  L  V  L          36
GCC CGG CGC CTG CTG GCC TCC GCC TCG TGG GTA CCC TGC ATA GTG CTG GGG CTG GTG CTG     108

 S  S  E  E  L  L  T  A  Q  P  A  P  H  C  R  P  D  P  T  L          56
AGC TCC GAG GAG CTG CTT ACC GCG CAG CCC GCG CCC CAC TGC CGA CCG GAC CCC ACG CTG     168

 L  P  P  A  L  R  A  L  R  G  P  A  L  L  D  A  A  I  P  R          76
TTG CCC CCA GCG CTG CGC GCC CTG CGC GGA CCC GCG CTG CTG GAC GCC GCC ATC CCG CGC     228

 L  G  P  T  R  A  A  S  P  C  L  L  L  R  Y  P  D  P  A  P          96
CTG GGG CCC ACG CGA GCC GCG AGC CCC TGC CTG CTC CTG CGC TAC CCC GAT CCC GCG CCC     288

 C  T  R  P  G  P  R  P  A  P  A  R  N  G  T  R  P  C  T  R         116
TGC ACC CGC CCC GGC CCG CGC CCC GCG CCC GCA CGC AAC GGC ACC CGG CCC TGC ACA CGC     348

 G  W  L  Y  A  L  P  G  A  G  L  L  Q  S  P  V  T  Q  W  N         136
GGC TGG CTC TAC GCG CTG CCC GGC GCC GGC CTC CTG CAA AGC CCG GTC ACC CAG TGG AAC     408

 L  V  C  G  D  G  W  K  V  P  L  E  Q  V  S  H  L  L  G  W         156
CTT GTG TGT GGA GAC GGC TGG AAG GTC CCG CTG GAG CAG GTG AGC CAC CTC CTG GGC TGG     468

 L  L  G  C  V  I  L  G  A  G  C  D  R  F  G  R  R  A  V  F         176
CTG CTG GGC TGT GTC ATC CTG GGA GCA GGC TGT GAC CGG TTT GGA CGC CGG GCA GTT TTT     528

 V  A  S  L  V  L  T  T  G  L  G  A  S  E  A  L  A  A  S  F         196
GTG GCC TCC CTG GTG CTG ACC ACA GGC CTG GGG GCC AGT GAG GCC CTG GCT GCC AGC TTC     588

 P  T  L  L  V  L  R  L  L  H  G  G  T  L  A  G  A  L  L  A         216
CCT ACC CTG CTG GTC CTG CGC CTA CTC CAC GGG GGC ACA TTG GCA GGG GCC CTC CTC GCC     648

 L  Y  L  A  R  L  E  L  C  D  P  P  H  R  L  A  F  S  M  G         236
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 D  W  R  L  L  Q  G  L  G  A  L  M  S  G  L  L  L  L  F  W         276
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 G  F  P  A  L  F  P  E  S  P  C  W  L  L  A  T  G  Q  V  A         296
GGG TTC CCG GCC CTG TTC CCC GAG TCT CCC TGC TGG CTG CTG GCC ACA GGT CAG GTA GCT     888

 R  A  R  K  I  L  W  R  F  A  E  A  S  G  V  D  P  G  D  S         316
CGA GCC AGG AAG ATC CTG TGG CGC TTT GCA GAA GCC AGT GGC GTG GAT CCC GGG GAC AGT     948

 P  L  E  E  N  S  L  A  T  E  L  T  M  L  S  A  R  S  P  Q         336
CCC TTG GAG GAG AAC TCC CTG GCT ACA GAG CTG ACC ATG CTG TCT GCA CGG AGC CCC CAG    1008

 P  R  Y  H  S  P  L  G  L  L  R  T  R  V  T  W  R  N  G  L         356
CCC CGG TAC CAC TCC CCA CTG GGG CTT CTG CGT ACC CGA GTC ACC TGG AGA AAC GGG CTT    1068

 I  L  G  F  S  S  L  V  G  G  G  I  R  A  S  F  R  R  S  L         376
ATC TTG GGC TTC AGC TCG CTG GTT GGT GGA GGC ATC AGA GCT AGC TTC CGC CGC AGC CTG    1128
```

Figure 1A

```
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 A  L  V  F  L  L  L  T  A  D  C  C  G  R  R  P  V  L  L  L   416
GCC TTG GTC TTC CTG CTC CTG ACG GCA GAT TGC TGT GGA CGC CGC CCC GTG CTG CTG CTG  1248

 G  T  M  V  T  G  L  A  S  L  L  L  L  A  G  A  Q  Y  L  P   436
GGC ACC ATG GTC ACA GGC CTG GCA TCC CTG CTG CTC CTC GCT GGG GCC CAG TAT CTG CCA  1308

 G  W  T  V  L  F  L  S  V  L  G  L  L  A  S  R  A  V  S  A   456
GGC TGG ACT GTG CTG TTC CTC TCT GTC CTG GGG CTC CTG GCC TCC CGG GCT GTG TCC GCA  1368

 L  S  S  L  F  A  A  E  V  F  P  T  V  I  R  G  A  G  L  G   476
CTC AGC AGC CTC TTC GCG GCC GAG GTC TTC CCC ACG GTG ATC AGG GGG GCC GGG CTG GGC  1428

 L  V  L  G  A  G  P  L  G  Q  A  A  G  P  L  D  T  L  H  G   496
CTG GTG CTG GGG GCC GGG TTC CTG GGC CAG GCA GCC GGC CCC CTG GAC ACC CTG CAC GGC  1488

 R  Q  G  F  F  L  Q  Q  V  V  F  A  S  L  A  V  L  A  L  L   516
CGG CAG GGC TTC TTC CTG CAA CAA GTC GTC TTC GCC TCC CTT GCT GTC CTT GCC CTG CTG  1548

 C  V  L  L  L  P  E  S  R  S  R  G  L  P  Q  S  L  Q  D  A   536
TGT GTC CTG CTG CTG CCT GAG AGC CGA AGC CGG GGG CTG CCC CAG TCA CTG CAG GAC GCC  1608

 D  R  L  R  R  P  P  L  L  R  G  R  P  R  Q  D  H  L  P  L   556
GAC CGC CTG CGC CGC CCC CCA CTC CTG CGG GGC CGC CCC CGC CAG GAC CAC CTG CCT CTG  1668

 L  P  P  S  N  S  Y  W  A  G  H  T  P  E  Q  H  *            573
CTG CCG CCC TCC AAC TCC TAC TGG GCC GGC CAC ACC CCC GAG CAG CAC TAG              1719

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CCAGGGCAGACACATTCCTCTCAGAAGCCCGTGTCTCAGTGCAGGTGGAGCCGTGGGGACAGCGTGAAGGTGTCTCCAG

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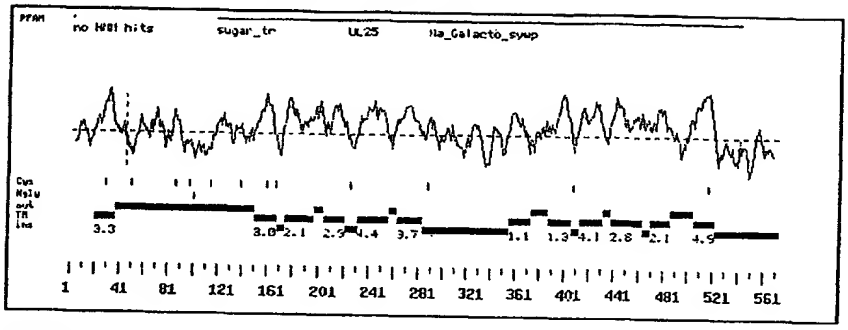
Figure 1B

Analysis of 57250.prot (572 aa)



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VLTTGLGASEALAASFPTLLVLRLLHGGTLAGALLALYLARLELCDPPHRLLAFSWGAGLF
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AGFLGQAAGPLDTLHGRQGFFLQGVVFASLAVLALLCVLLLPESRSRGLPQSLQDADRLR
RPPLLRGRPRQDHLPLLPPSNSYWAGHTPEQK

Figure 2

Protein Family / Domain Matches, HMMer version 2

```
Searching for complete domains in PFAM
hmmpfam - search a single seq against HMM database
HMMER 2.1.1 (Dec 1998)
Copyright (C) 1992-1998 Washington University School of Medicine
HMMER is freely distributed under the GNU General Public License (GPL).
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
HMM file:                 /prod/ddn/seqanal/PFAM/pfam5.3/Pfam
Sequence file:            /prod/ddn/wspace/orfanal/oa-script.7162.seq
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
  Query:  57250.prot

Scores for sequence family classification (score includes all domains):
Model            Description                                   Score    E-value  N
--------         -----------                                   -----    -------- ---
UL25             Herpesvirus UL25 family                         3.0        2.2  1
sugar_tr         Sugar (and other) transporter                 -53.5    6.1e-05  1
Na_Galacto_symp  Sodium:galactoside symporter family          -154.5        9.1  1

Parsed for domains:
Model            Domain  seq-f seq-t    hmm-f hmm-t       score  E-value
--------         ------- ----- -----    ----- -----       -----  -------
UL25               1/1     222   242 ..   577   597 .]      3.0        2.2
sugar_tr           1/1     117   536 ..     1   487 []    -53.5    6.1e-05
Na_Galacto_symp    1/1     287   541 ..     1   285 []  -154.5        9.1

Alignments of top-scoring domains:
UL25: domain 1 of 1, from 222 to 242: score 3.0, E = 2.2
                 *->LsqpsPrtaLareiisQPnV<-*
                    L+  +P+++La+ +  ++F+V
  57250.prot   222    LELCDPPHRLAFSHGAGLPSV    242

sugar_tr: domain 1 of 1, from 117 to 536: score -53.5, E = 6.1e-05
                 *->valvaalgQgflEGyDtgvigyflalidflfrfgllts=galaelgy
                       l a  g          g++ +++++ +      g+++  ++
  57250.prot   117    GWLYALPGA--------GLLQSPVTQWNLVCGDGWKVPLEQ------ 149

                    stvltglvvsifflGrliGslfaGklgdrfGRkkallialvlfvlGalls
                          + +lG l+G+++G+ +drfGR+  + ++lvl    ++
  57250.prot   150 ---------VSHLLGWLLGCVILGAGCDRFGRRAVFVASLVLTTGLGASE 190

                    gaapgytTiGlwafyllivGRvlvGlgvGgasvlvPmYis..ElA.Pkal
                     ++a         +f  l+v R+l G    +a +l ++Y+++ E ++P++
  57250.prot   191 ALAA--------SFPTLLVLRLLHG--GTLAGALLALYLArlELCdPPHR 230

                    RGalgslyqlaitiGilvAaiglgl.nktnndsalnswgWRiplglqlv
                      a  ++  l+ ++G l+      gl +++ +        WR++ gl+++
  57250.prot   231 L-APSMGAGLFSVVGTLL-----LPGLaALVGD--------WRLLQGLGAL 267

                    palllligl...lflPESPRwLvskgklseArevLak...lrgvedvdqe
                    +++lll++++ + + PESP wL++ g++ +Ar++L++  +  gv++ d
  57250.prot   268 MSGLLLLFWgfpALPPESPCWLLATGQVARARKILNRfaeASGVDPGDSP 317

                    iqeikaeleagvsee...kagkaswgelfrgrtrpkvrqr..llagvalq
                    +++e  +++ ++ + ++++   +s ++l+r+    +v +r++l++g+
  57250.prot   318 LEENSLATELTMLSArspQPRYHSPLGLLRT----RVTWRngLILGFSSL 363

                    afqQltGiNalfYYsptifksvGvsdsrasllvtiiivgvvNifvfTlvali
                    ++          ++ +s+  + ++  +l  ++ +++ +++ ++   +
  57250.prot   364 VGG----------GIRASFRRSLAPQVPTP-YLPYPLEAGLEAAALVFL-L 402

                    flvDrfGRRpllllGaagmaicflilgasigvalllinkpkdplskaagi
                     + D +GRRp lllG  ++ ++ l+l      + + +l +           +
  57250.prot   403 LTADCGRRPVLLLGTMVTGLASLLL----AGAQYLPGW------TVLF 442

                    vaivfillfiafFalgwGpipwvilsElFPtkvRskalalataanwlanf
                    +++ ++l   a+ al++      ++ +E+FPt +R+++l+l  +a++l+++
  57250.prot   443 LSVLGLLASRAVSALSSL----FA-AEVFPTVIRGAGLGLVLGAGFLGQA 487

                    iigflfpyitgaiglalggyvflvfagllvlfilfvfffvPETKGrtLee
                    + ++ +    +        +   vfa l+vl+   + +++PE + r L +
  57250.prot   488 AGPLDTLHGRGGF-----FLGQVVFASLAVLAL-LCVLLLPESRSRGLPQ 531

                    ieelf<-*
                      ++
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Na_Galacto_symp: domain 1 of 1, from 287 to 541: score -154.5, E = 9.1
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                          +++l  +++++    ++  f  +E   ++ + +++    sl+
  57250.prot   287    -----CWLLATGQVARARKILWRF--AEASGVDPG-DSPLEENSLAT 325

                    slklla....kNdQLliLclaa.....lfyllainilggaqlYYvtY
                     l  l  ++++++    L+l+ ++ + +++l ++ +  + gy+   + +
  57250.prot   326 ELTMLSarspQPRYHSPLGLLRtrvtwrngLILGFSSLVGGGIRASFRRS 375

                    vLGdpelFsyllynilvgligslLfPrLvkrfgkktvFagcivlmvlga
                          F + +++  ++ ++++++   ++++ g++ v + +  + +l
  57250.prot   376 LAPQVPTFYLFYFLEAGLEAAALVFLLLTADCCGRRPVLLLGTMVTGLA- 424

                    lliFfvagssl..alilvliflagilqqlvtllvWvlQVIMvsDtVDYGE
                    +l+++ +++ l+++++l l++l +  + +v +l  +      ++ +
  57250.prot   425 SLLLLAGAQYLpgWTVLFLSVLGLLASRAVSALSSLF----AAEV------ 465

                    wktGvRlEGlvySvflfvlKlGlAlsGalvGwiLgyiGYvanasqststa
                      t +R  Gl          Gl l+++ G+ +g              t+
  57250.prot   466 FPTVIRGAGL----------GLVLGAGFLGQAAGPL----------DTL 494

                    lgQlvfilalPalPpallllaafimlrfYkLtakklaaIveeLekWrtrk
                    +g    ++  F   ++++la+++ l+   L e + +     L     +r
  57250.prot   495 HG----RQGFFLQQVVFASLAVLALLCVLLLPESRSRGLPQSLQ-DADRL 539

                    rk<-*
                    r+
  57250.prot   540 RR    541
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Figure 3

Transmembrane Segments Predicted by MEMSAT

Start	End	Orient	Score
20	36	ins->out	3.3
150	167	out->ins	3.0
174	196	ins->out	2.1
204	220	out->ins	2.9
231	255	ins->out	4.4
263	282	out->ins	3.7
355	372	ins->out	1.1
387	405	out->ins	1.3
413	431	ins->out	4.1
438	462	out->ins	2.8
469	485	ins->out	2.1
505	521	out->ins	4.9

>57250.prot
MEQEARVLRAAGGFGSARRLLASASWVPCIVLGLVLSSEELLTAQPARHCRFDPTLLPPA
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VLTPVQLGKSRALAASFPTLLVLRLLHGGTLAGALLALYLARLELCDPPHRLASPHGAGLF
SVVGTLLLPGLAALVQDMRLLQGLGALNSQLLLLFHSPPALFPESPCWLLATGQVARARK

Figure 4

```
CLUSTAL W (1.74) multiple sequence alignment

AB040056        ----------------------------------MASDPIFTLAPPLHCHYG--ALPPN
Fbh57250        MEQEARVLRAAGGFGRARRLLASASWVPCIVLGLVLSSEELLTAQPAPHCRPDPTLLPPA
                                                  ::*: ::*  *. **: .   ***

AB040056        ASGWEQPPN-SSGVSVAGAALAASAASRVVTSTDPS-CS--GFAP-PDFN---HCLKDWD
Fbh57250        LRALRGPALLDAAIPRLGPTRAASP-CLLLRYPDPAPCTRPGPRPAPARNGTRPCTRGWL
                  . . *.  .:.:.  *.: ***. . ::  .**: *:  *  * *  *    * :.*

AB040056        YNGLP--VLTTNAIGQWDLVCDLGWQVILEQILFILGFASGYLFLGYPADRFGRRGIVLL
Fbh57250        Y-ALPGAGLLQSPVTQWNLVCGDGWKVPLEQVSHLLGWLLGCVILGAGCDRFGRRAVFVA
                * .**   *  ..: **:***. **:* ***: .:**:  * ::**  .******.:.:

AB040056        TLGLVGPCGVGGAAAGSSTGIMTLRFLLGFLLAGVDLGVYLMRLELCDPTQRLRVALAGE
Fbh57250        SLVLTTGLGASEALAASFPTLLVLRLLHGGTLAGALLALYLARLELCDPPHRLAFSMGAG
                :* *.   *.. * *.* . ::.**:* *  ***. *.:** *******.:** .::..

AB040056        LVGVGGHFLFLGLALVSKDWRFLQRM-ITAPCILFLFYGWPGLFLESARWLIVKRQIEEA
Fbh57250        LFSVVGTLLLPGLAALVQDWRLLQGLGALMSGLLLLFWGFPALFPESPCWLLATGQVARA
                *..* * :*: *** : :***:** :     . :*:**:*:*.** **._**:.. *: .*

AB040056        QSVLRILAERN-RPHGQMLGEEAQEALQELENTCPLPTTSTFSFASLLNYRNIWKNLLIL
Fbh57250        RKILWRFAEASGVDPGDSPLEENSLATELTMLSARSPQPRYHSPLGLLRTRVTWRNGLIL
                :.:*  :** .    *:   ** . * :    :.  * .  .*  .**. *  *:* ***

AB040056        GFTNFIAHAIRHCYQPVGGGGSPSDFYLCSLLASGTAALACVFLGVTVDRFGRRGILLLS
Fbh57250        GFSSLVGGGIRASFRRSLAPQVPT-FYLPYFLEAGLEAAALVFLLLTADCCGRRPVLLLG
                **:.::. .** .::    .   *: ***  :* :*  * * *** :*.*  *** :***.

AB040056        MTLTGIASLVLLGLWDYLNDAAITTFSVLGLFSSQASAILSTLLAAEVIPTTVRGRGLGL
Fbh57250        TMVTGLASLLLLAGAQYLPGWTVLFLSVLGLLASRAVSALSSLFAAEVFPTVIRGAGLGL
                  :**:***:**.  :** . ::  :*****::*:* : **:*:****:**.:** ****

AB040056        IMALGALGGLSCPAQRLHMGHGAFLQHVVLAACALLCILSIMLLPETKRKLLPEVLRDGE
Fbh57250        VLGAGFLGQAAGPLDTLHGRQGFFLQQVVFASLAVLALLCVLLLPESRSRGLPQSLQDAD
                ::. * **  : * : **  :* ***:**:*:.*:*.:*.::****:: : **: *:*.:

AB040056        LCRRPSLLRQPPPNRCDHVPLLATS------------
Fbh57250        RLRRPPLLRGRP--RQDHLPLLPPSNSYWAGHTPEQH
                  ***.***  *  * **:***..*
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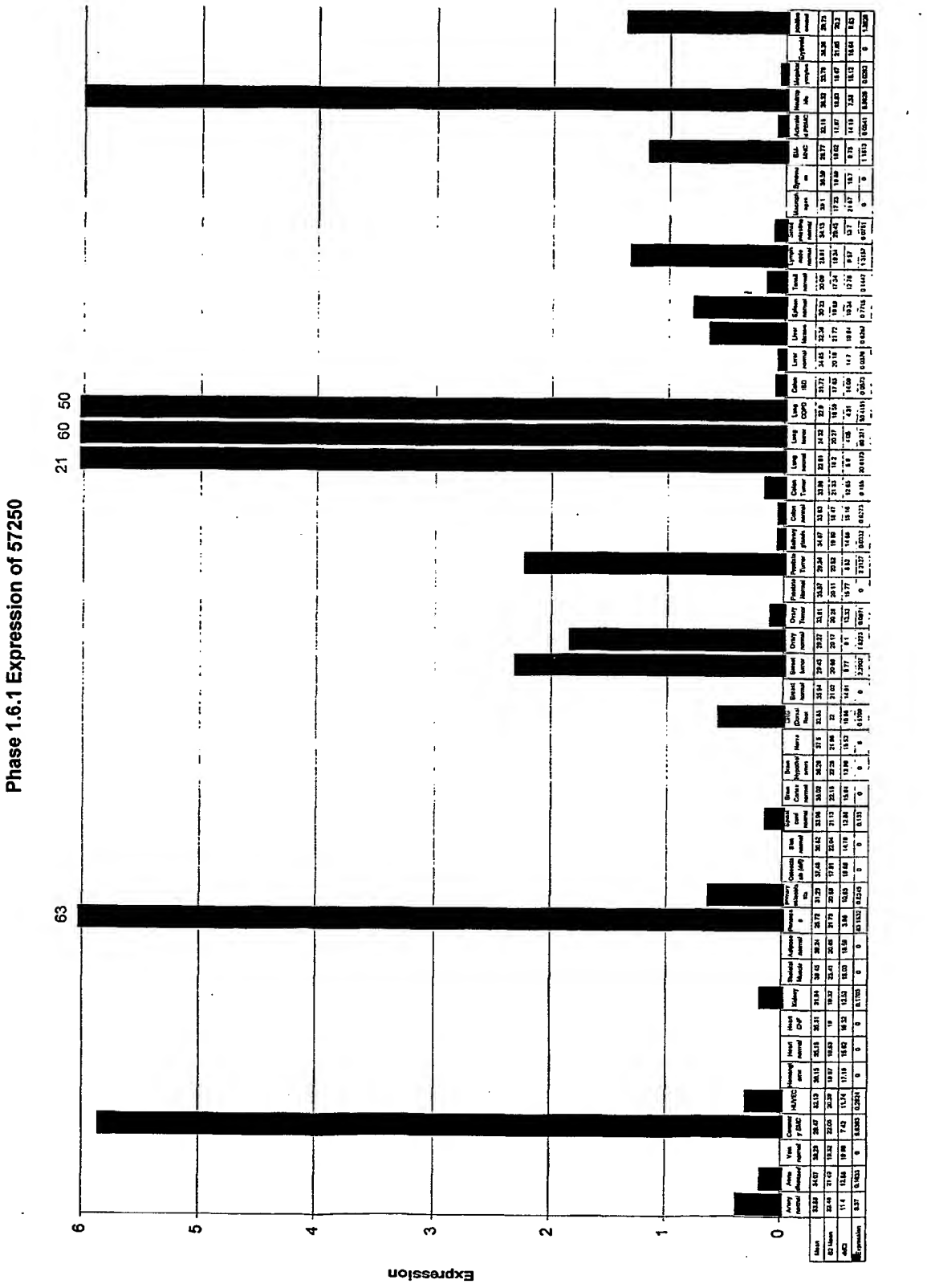
Figure 5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Expression
0
1
2
3
4
5
6
63
21
60
50

Figure 6

SEQUENCE LISTING

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1 5 10

ttt ggc cgg gcc cgg cgc ctg ctg gcc tcc gcc tcg tgg gta ccc tgc 99
Phe Gly Arg Ala Arg Arg Leu Leu Ala Ser Ala Ser Trp Val Pro Cys
15 20 25

ata gtg ctg ggg ctg gtg ctg agc tcc gag gag ctg ctt acc gcg cag 147
Ile Val Leu Gly Leu Val Leu Ser Ser Glu Glu Leu Leu Thr Ala Gln
30 35 40 45

ccc gcg ccc cac tgc cga ccg gac ccc acg ctg ttg ccc cca gcg ctg 195
Pro Ala Pro His Cys Arg Pro Asp Pro Thr Leu Leu Pro Pro Ala Leu
50 55 60

cgc gcc ctg cgc gga ccc gcg ctg ctg gac gcc gcc atc ccg cgc ctg 243
Arg Ala Leu Arg Gly Pro Ala Leu Leu Asp Ala Ala Ile Pro Arg Leu
65 70 75

ggg ccc acg cga gcc gcg agc ccc tgc ctg ctc ctg cgc tac ccc gat 291
Gly Pro Thr Arg Ala Ala Ser Pro Cys Leu Leu Leu Arg Tyr Pro Asp
80 85 90

ccc gcg ccc tgc acc cgc ccc ggc ccg cgc ccc gcg ccc gca cgc aac 339
Pro Ala Pro Cys Thr Arg Pro Gly Pro Arg Pro Ala Pro Ala Arg Asn
95 100 105

ggc acc cgg ccc tgc aca cgc ggc tgg ctc tac gcg ctg ccc ggc gcc 387
Gly Thr Arg Pro Cys Thr Arg Gly Trp Leu Tyr Ala Leu Pro Gly Ala
110 115 120 125

ggc ctc ctg caa agc ccg gtc acc cag tgg aac ctt gtg tgt gga gac 435
Gly Leu Leu Gln Ser Pro Val Thr Gln Trp Asn Leu Val Cys Gly Asp
130 135 140

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            145                 150                 155

ctg ggc tgt gtc atc ctg gga gca ggc tgt gac cgg ttt gga cgc cgg   531
Leu Gly Cys Val Ile Leu Gly Ala Gly Cys Asp Arg Phe Gly Arg Arg
        160                 165                 170

gca gtt ttt gtg gcc tcc ctg gtg ctg acc aca ggc ctg ggg gcc agt   579
Ala Val Phe Val Ala Ser Leu Val Leu Thr Thr Gly Leu Gly Ala Ser
    175                 180                 185

gag gcc ctg gct gcc agc ttc cct acc ctg ctg gtc ctg cgc cta ctc   627
Glu Ala Leu Ala Ala Ser Phe Pro Thr Leu Leu Val Leu Arg Leu Leu
190                 195                 200                 205

cac ggg ggc aca ttg gca ggg gcc ctc ctc gcc ctg tat ctg gct cgc   675
His Gly Gly Thr Leu Ala Gly Ala Leu Leu Ala Leu Tyr Leu Ala Arg
                210                 215                 220

ctg gag ttg tgt gac cct ccc cac cgc ctg gcc ttc tcc atg ggg gct   723
Leu Glu Leu Cys Asp Pro Pro His Arg Leu Ala Phe Ser Met Gly Ala
            225                 230                 235

ggc ctt ttc tcg gtg gtg ggc acc ctg ctg ctg ccc ggc ctg gct gcg   771
Gly Leu Phe Ser Val Val Gly Thr Leu Leu Leu Pro Gly Leu Ala Ala
        240                 245                 250

ctt gtg cag gac tgg cgt ctt ctg cag ggg ctg ggc gcc ctg atg agt   819
Leu Val Gln Asp Trp Arg Leu Leu Gln Gly Leu Gly Ala Leu Met Ser
    255                 260                 265

gga ctc ttg ctg ctc ttt tgg ggg ttc ccg gcc ctg ttc ccc gag tct   867
Gly Leu Leu Leu Leu Phe Trp Gly Phe Pro Ala Leu Phe Pro Glu Ser
270                 275                 280                 285

ccc tgc tgg ctg ctg gcc aca ggt cag gta gct cga gcc agg aag atc   915
Pro Cys Trp Leu Leu Ala Thr Gly Gln Val Ala Arg Ala Arg Lys Ile
                290                 295                 300

ctg tgg cgc ttt gca gaa gcc agt ggc gtg gat ccc ggg gac agt ccc   963
Leu Trp Arg Phe Ala Glu Ala Ser Gly Val Asp Pro Gly Asp Ser Pro
            305                 310                 315

ttg gag gag aac tcc ctg gct aca gag ctg acc atg ctg tct gca cgg   1011
Leu Glu Glu Asn Ser Leu Ala Thr Glu Leu Thr Met Leu Ser Ala Arg
        320                 325                 330

agc ccc cag ccc cgg tac cac tcc cca ctg ggg ctt ctg cgt acc cga   1059
Ser Pro Gln Pro Arg Tyr His Ser Pro Leu Gly Leu Leu Arg Thr Arg
    335                 340                 345

gtc acc tgg aga aac ggg ctt atc ttg ggc ttc agc tcg ctg gtt ggt   1107
Val Thr Trp Arg Asn Gly Leu Ile Leu Gly Phe Ser Ser Leu Val Gly
350                 355                 360                 365

gga ggc atc aga gct agc ttc cgc cgc agc ctg gca cct cag gtg ccg   1155
Gly Gly Ile Arg Ala Ser Phe Arg Arg Ser Leu Ala Pro Gln Val Pro
                370                 375                 380
```

```
acc ttc tac ctg ccc tac ttc ctg gag gcc ggc ctg gag gcg gca gcc   1203
Thr Phe Tyr Leu Pro Tyr Phe Leu Glu Ala Gly Leu Glu Ala Ala Ala
            385                 390                 395

ttg gtc ttc ctg ctc ctg acg gca gat tgc tgt gga cgc cgc ccc gtg   1251
Leu Val Phe Leu Leu Leu Thr Ala Asp Cys Cys Gly Arg Arg Pro Val
        400                 405                 410

ctg ctg ctg ggc acc atg gtc aca ggc ctg gca tcc ctg ctg ctc ctc   1299
Leu Leu Leu Gly Thr Met Val Thr Gly Leu Ala Ser Leu Leu Leu Leu
    415                 420                 425

gct ggg gcc cag tat ctg cca ggc tgg act gtg ctg ttc ctc tct gtc   1347
Ala Gly Ala Gln Tyr Leu Pro Gly Trp Thr Val Leu Phe Leu Ser Val
430                 435                 440                 445

ctg ggg ctc ctg gcc tcc cgg gct gtg tcc gca ctc agc agc ctc ttc   1395
Leu Gly Leu Leu Ala Ser Arg Ala Val Ser Ala Leu Ser Ser Leu Phe
                450                 455                 460

gcg gcc gag gtc ttc ccc acg gtg atc agg ggg gcc ggg ctg ggc ctg   1443
Ala Ala Glu Val Phe Pro Thr Val Ile Arg Gly Ala Gly Leu Gly Leu
            465                 470                 475

gtg ctg ggg gcc ggg ttc ctg ggc cag gca gcc ggc ccc ctg gac acc   1491
Val Leu Gly Ala Gly Phe Leu Gly Gln Ala Ala Gly Pro Leu Asp Thr
        480                 485                 490

ctg cac ggc cgg cag ggc ttc ttc ctg caa caa gtc gtc ttc gcc tcc   1539
Leu His Gly Arg Gln Gly Phe Phe Leu Gln Gln Val Val Phe Ala Ser
    495                 500                 505

ctt gct gtc ctt gcc ctg ctg tgt gtc ctg ctg ctg cct gag agc cga   1587
Leu Ala Val Leu Ala Leu Leu Cys Val Leu Leu Leu Pro Glu Ser Arg
510                 515                 520                 525

agc cgg ggg ctg ccc cag tca ctg cag gac gcc gac cgc ctg cgc cgc   1635
Ser Arg Gly Leu Pro Gln Ser Leu Gln Asp Ala Asp Arg Leu Arg Arg
                530                 535                 540

ccc cca ctc ctg cgg ggc cgc ccc cgc cag gac cac ctg cct ctg ctg   1683
Pro Pro Leu Leu Arg Gly Arg Pro Arg Gln Asp His Leu Pro Leu Leu
            545                 550                 555

ccg ccc tcc aac tcc tac tgg gcc ggc cac acc ccc gag cag cac       1728
Pro Pro Ser Asn Ser Tyr Trp Ala Gly His Thr Pro Glu Gln His
        560                 565                 570

tagtcctgcc tggtggccct gggagccagg atgggaccaa agtcaaggcc tggggcatgg 1788
ctgagtaccc cagacgtctg gtccagggca gacacattcc tctcagaagc ccgtgtctca 1848
gtgcaggtgg agccgtgggg acagcgtgaa ggtgtctcca gccaggcccc aggcactggg 1908
aggccctgg                                                         1917
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<210> 2
<211> 572
<212> PRT
<213> Homo sapiens

<400> 2

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Met Glu Gln Glu Ala Arg Val Leu Arg Ala Ala Gly Gly Phe Gly Arg
 1               5                  10                  15
Ala Arg Arg Leu Leu Ala Ser Ala Ser Trp Val Pro Cys Ile Val Leu
            20                  25                  30
Gly Leu Val Leu Ser Ser Glu Glu Leu Leu Thr Ala Gln Pro Ala Pro
        35                  40                  45
His Cys Arg Pro Asp Pro Thr Leu Leu Pro Pro Ala Leu Arg Ala Leu
    50                  55                  60
Arg Gly Pro Ala Leu Leu Asp Ala Ala Ile Pro Arg Leu Gly Pro Thr
65                  70                  75                  80
Arg Ala Ala Ser Pro Cys Leu Leu Leu Arg Tyr Pro Asp Pro Ala Pro
                85                  90                  95
Cys Thr Arg Pro Gly Pro Arg Pro Ala Pro Ala Arg Asn Gly Thr Arg
            100                 105                 110
Pro Cys Thr Arg Gly Trp Leu Tyr Ala Leu Pro Gly Ala Gly Leu Leu
        115                 120                 125
Gln Ser Pro Val Thr Gln Trp Asn Leu Val Cys Gly Asp Gly Trp Lys
    130                 135                 140
Val Pro Leu Glu Gln Val Ser His Leu Leu Gly Trp Leu Leu Gly Cys
145                 150                 155                 160
Val Ile Leu Gly Ala Gly Cys Asp Arg Phe Gly Arg Arg Ala Val Phe
                165                 170                 175
Val Ala Ser Leu Val Leu Thr Thr Gly Leu Gly Ala Ser Glu Ala Leu
            180                 185                 190
Ala Ala Ser Phe Pro Thr Leu Leu Val Leu Arg Leu Leu His Gly Gly
        195                 200                 205
Thr Leu Ala Gly Ala Leu Leu Ala Leu Tyr Leu Ala Arg Leu Glu Leu
    210                 215                 220
Cys Asp Pro Pro His Arg Leu Ala Phe Ser Met Gly Ala Gly Leu Phe
225                 230                 235                 240
Ser Val Val Gly Thr Leu Leu Leu Pro Gly Leu Ala Ala Leu Val Gln
                245                 250                 255
Asp Trp Arg Leu Leu Gln Gly Leu Gly Ala Leu Met Ser Gly Leu Leu
            260                 265                 270
Leu Leu Phe Trp Gly Phe Pro Ala Leu Phe Pro Glu Ser Pro Cys Trp
        275                 280                 285
Leu Leu Ala Thr Gly Gln Val Ala Arg Ala Arg Lys Ile Leu Trp Arg
    290                 295                 300
Phe Ala Glu Ala Ser Gly Val Asp Pro Gly Asp Ser Pro Leu Glu Glu
305                 310                 315                 320
Asn Ser Leu Ala Thr Glu Leu Thr Met Leu Ser Ala Arg Ser Pro Gln
                325                 330                 335
Pro Arg Tyr His Ser Pro Leu Gly Leu Leu Arg Thr Arg Val Thr Trp
            340                 345                 350
Arg Asn Gly Leu Ile Leu Gly Phe Ser Ser Leu Val Gly Gly Gly Ile
        355                 360                 365
Arg Ala Ser Phe Arg Arg Ser Leu Ala Pro Gln Val Pro Thr Phe Tyr
    370                 375                 380
Leu Pro Tyr Phe Leu Glu Ala Gly Leu Glu Ala Ala Ala Leu Val Phe
385                 390                 395                 400
Leu Leu Leu Thr Ala Asp Cys Cys Gly Arg Arg Pro Val Leu Leu Leu
                405                 410                 415
Gly Thr Met Val Thr Gly Leu Ala Ser Leu Leu Leu Leu Ala Gly Ala
            420                 425                 430
```

```
Gln Tyr Leu Pro Gly Trp Thr Val Leu Phe Leu Ser Val Leu Gly Leu
        435                 440                 445
Leu Ala Ser Arg Ala Val Ser Ala Leu Ser Ser Leu Phe Ala Ala Glu
    450                 455                 460
Val Phe Pro Thr Val Ile Arg Gly Ala Gly Leu Gly Leu Val Leu Gly
465                 470                 475                 480
Ala Gly Phe Leu Gly Gln Ala Ala Gly Pro Leu Asp Thr Leu His Gly
                485                 490                 495
Arg Gln Gly Phe Phe Leu Gln Gln Val Val Phe Ala Ser Leu Ala Val
            500                 505                 510
Leu Ala Leu Leu Cys Val Leu Leu Leu Pro Glu Ser Arg Ser Arg Gly
        515                 520                 525
Leu Pro Gln Ser Leu Gln Asp Ala Asp Arg Leu Arg Arg Pro Pro Leu
    530                 535                 540
Leu Arg Gly Arg Pro Arg Gln Asp His Leu Pro Leu Leu Pro Pro Ser
545                 550                 555                 560
Asn Ser Tyr Trp Ala Gly His Thr Pro Glu Gln His
                565                 570
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<210> 3
<211> 1716
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(1716)

<400> 3

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atg gag cag gag gcg cgg gtg ctg aga gct gcg ggc ggc ttt ggc cgg   48
Met Glu Gln Glu Ala Arg Val Leu Arg Ala Ala Gly Gly Phe Gly Arg
 1               5                  10                  15

gcc cgg cgc ctg ctg gcc tcc gcc tcg tgg gta ccc tgc ata gtg ctg   96
Ala Arg Arg Leu Leu Ala Ser Ala Ser Trp Val Pro Cys Ile Val Leu
            20                  25                  30

ggg ctg gtg ctg agc tcc gag gag ctg ctt acc gcg cag ccc gcg ccc   144
Gly Leu Val Leu Ser Ser Glu Glu Leu Leu Thr Ala Gln Pro Ala Pro
        35                  40                  45

cac tgc cga ccg gac ccc acg ctg ttg ccc cca gcg ctg cgc gcc ctg   192
His Cys Arg Pro Asp Pro Thr Leu Leu Pro Pro Ala Leu Arg Ala Leu
    50                  55                  60

cgc gga ccc gcg ctg ctg gac gcc gcc atc ccg cgc ctg ggg ccc acg   240
Arg Gly Pro Ala Leu Leu Asp Ala Ala Ile Pro Arg Leu Gly Pro Thr
65                  70                  75                  80

cga gcc gcg agc ccc tgc ctg ctc ctg cgc tac ccc gat ccc gcg ccc   288
Arg Ala Ala Ser Pro Cys Leu Leu Leu Arg Tyr Pro Asp Pro Ala Pro
                85                  90                  95

tgc acc cgc ccc ggc ccg cgc ccc gcg ccc gca cgc aac ggc acc cgg   336
Cys Thr Arg Pro Gly Pro Arg Pro Ala Pro Ala Arg Asn Gly Thr Arg
            100                 105                 110

ccc tgc aca cgc ggc tgg ctc tac gcg ctg ccc ggc gcc ggc ctc ctg   384
Pro Cys Thr Arg Gly Trp Leu Tyr Ala Leu Pro Gly Ala Gly Leu Leu
        115                 120                 125
```

```
caa agc ccg gtc acc cag tgg aac ctt gtg tgt gga gac ggc tgg aag   432
Gln Ser Pro Val Thr Gln Trp Asn Leu Val Cys Gly Asp Gly Trp Lys
    130                 135                 140

gtc ccg ctg gag cag gtg agc cac ctc ctg ggc tgg ctg ctg ggc tgt   480
Val Pro Leu Glu Gln Val Ser His Leu Leu Gly Trp Leu Leu Gly Cys
145                 150                 155                 160

gtc atc ctg gga gca ggc tgt gac cgg ttt gga cgc cgg gca gtt ttt   528
Val Ile Leu Gly Ala Gly Cys Asp Arg Phe Gly Arg Arg Ala Val Phe
                165                 170                 175

gtg gcc tcc ctg gtg ctg acc aca ggc ctg ggg gcc agt gag gcc ctg   576
Val Ala Ser Leu Val Leu Thr Thr Gly Leu Gly Ala Ser Glu Ala Leu
            180                 185                 190

gct gcc agc ttc cct acc ctg ctg gtc ctg cgc cta ctc cac ggg ggc   624
Ala Ala Ser Phe Pro Thr Leu Leu Val Leu Arg Leu Leu His Gly Gly
        195                 200                 205

aca ttg gca ggg gcc ctc ctc gcc ctg tat ctg gct cgc ctg gag ttg   672
Thr Leu Ala Gly Ala Leu Leu Ala Leu Tyr Leu Ala Arg Leu Glu Leu
    210                 215                 220

tgt gac cct ccc cac cgc ctg gcc ttc tcc atg ggg gct ggc ctt ttc   720
Cys Asp Pro Pro His Arg Leu Ala Phe Ser Met Gly Ala Gly Leu Phe
225                 230                 235                 240

tcg gtg gtg ggc acc ctg ctg ctg ccc ggc ctg gct gcg ctt gtg cag   768
Ser Val Val Gly Thr Leu Leu Leu Pro Gly Leu Ala Ala Leu Val Gln
                245                 250                 255

gac tgg cgt ctt ctg cag ggg ctg ggc gcc ctg atg agt gga ctc ttg   816
Asp Trp Arg Leu Leu Gln Gly Leu Gly Ala Leu Met Ser Gly Leu Leu
            260                 265                 270

ctg ctc ttt tgg ggg ttc ccg gcc ctg ttc ccc gag tct ccc tgc tgg   864
Leu Leu Phe Trp Gly Phe Pro Ala Leu Phe Pro Glu Ser Pro Cys Trp
        275                 280                 285

ctg ctg gcc aca ggt cag gta gct cga gcc agg aag atc ctg tgg cgc   912
Leu Leu Ala Thr Gly Gln Val Ala Arg Ala Arg Lys Ile Leu Trp Arg
    290                 295                 300

ttt gca gaa gcc agt ggc gtg gat ccc ggg gac agt ccc ttg gag gag   960
Phe Ala Glu Ala Ser Gly Val Asp Pro Gly Asp Ser Pro Leu Glu Glu
305                 310                 315                 320

aac tcc ctg gct aca gag ctg acc atg ctg tct gca cgg agc ccc cag   1008
Asn Ser Leu Ala Thr Glu Leu Thr Met Leu Ser Ala Arg Ser Pro Gln
                325                 330                 335

ccc cgg tac cac tcc cca ctg ggg ctt ctg cgt acc cga gtc acc tgg   1056
Pro Arg Tyr His Ser Pro Leu Gly Leu Leu Arg Thr Arg Val Thr Trp
            340                 345                 350

aga aac ggg ctt atc ttg ggc ttc agc tcg ctg gtt ggt gga ggc atc   1104
Arg Asn Gly Leu Ile Leu Gly Phe Ser Ser Leu Val Gly Gly Gly Ile
        355                 360                 365
```

```
aga gct agc ttc cgc cgc agc ctg gca cct cag gtg ccg acc ttc tac   1152
Arg Ala Ser Phe Arg Arg Ser Leu Ala Pro Gln Val Pro Thr Phe Tyr
    370                 375                 380

ctg ccc tac ttc ctg gag gcc ggc ctg gag gcg gca gcc ttg gtc ttc   1200
Leu Pro Tyr Phe Leu Glu Ala Gly Leu Glu Ala Ala Ala Leu Val Phe
385                 390                 395                 400

ctg ctc ctg acg gca gat tgc tgt gga cgc cgc ccc gtg ctg ctg ctg   1248
Leu Leu Leu Thr Ala Asp Cys Cys Gly Arg Arg Pro Val Leu Leu Leu
                405                 410                 415

ggc acc atg gtc aca ggc ctg gca tcc ctg ctg ctc ctc gct ggg gcc   1296
Gly Thr Met Val Thr Gly Leu Ala Ser Leu Leu Leu Leu Ala Gly Ala
            420                 425                 430

cag tat ctg cca ggc tgg act gtg ctg ttc ctc tct gtc ctg ggg ctc   1344
Gln Tyr Leu Pro Gly Trp Thr Val Leu Phe Leu Ser Val Leu Gly Leu
        435                 440                 445

ctg gcc tcc cgg gct gtg tcc gca ctc agc agc ctc ttc gcg gcc gag   1392
Leu Ala Ser Arg Ala Val Ser Ala Leu Ser Ser Leu Phe Ala Ala Glu
    450                 455                 460

gtc ttc ccc acg gtg atc agg ggg gcc ggg ctg ggc ctg gtg ctg ggg   1440
Val Phe Pro Thr Val Ile Arg Gly Ala Gly Leu Gly Leu Val Leu Gly
465                 470                 475                 480

gcc ggg ttc ctg ggc cag gca gcc ggc ccc ctg gac acc ctg cac ggc   1488
Ala Gly Phe Leu Gly Gln Ala Ala Gly Pro Leu Asp Thr Leu His Gly
                485                 490                 495

cgg cag ggc ttc ttc ctg caa caa gtc gtc ttc gcc tcc ctt gct gtc   1536
Arg Gln Gly Phe Phe Leu Gln Gln Val Val Phe Ala Ser Leu Ala Val
            500                 505                 510

ctt gcc ctg ctg tgt gtc ctg ctg ctg cct gag agc cga agc cgg ggg   1584
Leu Ala Leu Leu Cys Val Leu Leu Leu Pro Glu Ser Arg Ser Arg Gly
        515                 520                 525

ctg ccc cag tca ctg cag gac gcc gac cgc ctg cgc cgc ccc cca ctc   1632
Leu Pro Gln Ser Leu Gln Asp Ala Asp Arg Leu Arg Arg Pro Pro Leu
    530                 535                 540

ctg cgg ggc cgc ccc cgc cag gac cac ctg cct ctg ctg ccg ccc tcc   1680
Leu Arg Gly Arg Pro Arg Gln Asp His Leu Pro Leu Leu Pro Pro Ser
545                 550                 555                 560

aac tcc tac tgg gcc ggc cac acc ccc gag cag cac                   1716
Asn Ser Tyr Trp Ala Gly His Thr Pro Glu Gln His
                565                 570
```